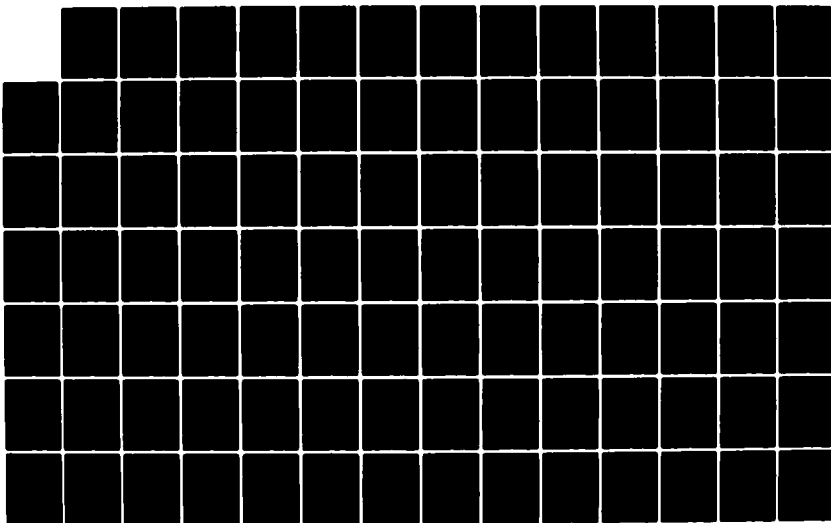AD-A130 671   COMPILATION OF 1982 ANNUAL REPORTS OF THE NAVY ELF   1/5
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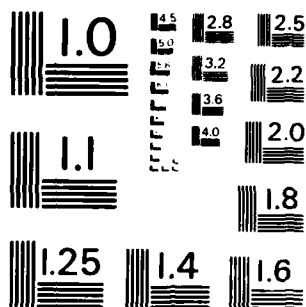   IL   MAY 83 IITRI-E06516-5 N00039-81-C-0357

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COMPILATION OF 1982 ANNUAL REPORTS OF THE
NAVY ELF COMMUNICATIONS SYSTEM
ECOLOGICAL MONITORING PROGRAM

May 1983

Prepared for:

U.S. Naval Electronic Systems Command
Communications Systems Project Office
Washington, D.C. 20360

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THIS PAPER IS A COMPONENT PART OF THE FOLLOWING COMPILATION REPORT:

(TITLE): Compilation of 1982 Annual Reports of the Navy ELF (Extremely Low Frequency) Communications System Ecological Monitoring Program.

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P001 276 ELF (Extremely Low Frequency) Communications System Ecological Monitoring Program. Litter Decomposition and Microflora.

P001 277 ELF (Extremely Low Frequency) Communications System Ecological Monitoring Program. The Effects of Exposing the Slime Mold Physarum polycephalum to Electromagnetic Fields.

P001 278 ELF (Extremely Low Frequency) Communications System Ecological Monitoring Program. Task 5.2, Soil Amoeba.

P001 279 ELF (Extremely Low Frequency) Communications System Ecological Monitoring Program. Soil and Litter Arthropoda and Earthworm Studies.

P001 280 ELF (Extremely Low Frequency) Communications System Ecological Monitoring Program. Biological Studies on Pollinating Insects: Megachilid Bees.

P001 281 ELF (Extremely Low Frequency) Communications System Ecological Monitoring Program. Small Vertebrates: The Michigan Study Site Tasks 5.6, Small Mammals, and %.12A, Nesting Birds.

P001 282 ELF (Extremely Low Frequency) Communications System Ecological Monitoring Program. Annual Report for Aquatic Ecosystems-Tasks 5.8, 5.9, 5.10.

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A long-term program of studying low-level ELF electromagnetic influences on eco-systems has been initiated in northwestern Wisconsin and the Upper Peninsula of Michigan. Plans are described for studying the terrestrial ecosystem, the aqua-tic ecosystem and the wetland ecosystem. Selection of study sites, monitoring protocols, and analytical methods were initiated in 1982, and data collection will commence in 1983.

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FOREWORD

This document is the first compilation of Annual Reports of the Ecological Monitoring Program - ELF Communications System authorized under IITRI Subcontract E06516-82-C-10015/40015 to Naval Electronic Systems Command Contract N00039-81-C-0357. These subcontracts include thirteen studies monitoring various ecosystem components in Northern Wisconsin and Michigan. Several studies have been grouped for cost and analytical efficiency. This initial compilation summarizes the proposed work and progress achieved during the period July 1982 - October 1982. Two additional studies (Wetland and Migrating Birds) will be funded during 1983.

The purpose of the Ecological Monitoring Program is to assess the influence of electromagnetic fields produced by the ELF Communications System on major ecosystem components in the ELF System area. Multi-year studies of both pre-operation and post-operation conditions are anticipated.

Each document in this volume was printed from an unedited copy of the principal investigators' annual report.
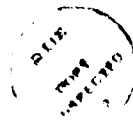
1982 Annual Reports - Ecological Monitoring Program

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ELF COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM*

HERBACEOUS PLANT COVER AND TREE STUDIES

The Michigan Study Site

Tasks 5.13/5.14 .

ANNUAL REPORT, 1982

SUBCONTRACT NUMBER: E06516-82-C-10015

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ABSTRACT

Since forest vegetation is dominant on the proposed ELF communications antenna area, it is essential to include plants in an ecological monitoring program. Trees and herbaceous plants having both above and below ground biomass will be more closely coupled to the electric field than those organisms solely in the air or on the soil surface. Trees differ from herbaceous plants in that they are more deeply rooted and are longer lived, while herbaceous plants are more sensitive to site disturbance than trees. However, trees offer the opportunity to evaluate effects of the ELF electro-magnetic field on the same individuals over a much longer period of time. These considerations would be of paramount importance in assessing the significance of ELF field effects on the plant community.

The objective of this study is to investigate and characterize the growth of trees and herbaceous plants on selected plots within the ELF antenna area prior to the actual operation of the communication system (1986). This information will be used as a baseline to evaluate possible ELF effects on the associated forest communities when the antenna becomes operable. In order to accomplish this goal, the monitoring of possible ELF effects on trees and herbaceous plants has been divided into seven study elements: (1) plot selection and statistical design, (2) ambient monitoring, (3) tree productivity, (4) tree phenology, (5) herbaceous plant growth, (6) root growth and mycorrhizal development, and (7) litter production.

The major activity during the initial four month period was the screening of forest stands for use as ELF study or control plots. Unfortunately, much of this work had to be accomplished prior to final selection of the ELF antenna site. Reconnaisance of ELF grid area showed a wide mosaic of forest conditions, topography and soil types. Final study site selection will not be possible until antenna placement is known, particularly the location of the ground terminals.

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INTRODUCTION

Since forest vegetation is dominant on the proposed ELF communications antenna area, it is essential to include it in an ecological monitoring program. However, there are several other considerations which justify this study. Trees and herbaceous plants having both above and below ground biomass will be more closely coupled to the electric field than those organisms solely in the air or on the soil surface (Anonymous, 1977). Trees differ from herbaceous plants in that they are more deeply rooted and are longer lived, while herbaceous plants have been found to be more sensitive to site disturbance than trees (National Research Council, 1977). However, trees offer the unique opportunity to evaluate effects of the ELF electromagnetic field on the same individuals over a much longer period of time while also evaluating changes in stand dynamics. These considerations would be of paramount importance in assessing the significance of ELF field effects occurring at the organismal level.

A secondary consideration is that forest vegetation also exerts strong influence on other organisms within the ecosystem, both above and below ground. These effects include modifying microclimate, exerting influence on soil organisms, particularly in the rhizosphere, and by influencing soil development and fertility through nutrient cycling. By studying the effects of ELF fields on individual plants and plants in the ecosystem, information gained will also be useful to investigators studying other ecological relationships in the ELF environmental monitoring program.

While there are many measures of tree response to any given stand treatment, only a few are generally needed to quantify that response and

test its significance. However, these measurements must be chosen on the basis of highest sensitivity, especially in the case of ELF field effects, since previous work has indicated these may be extremely subtle (National Research Council, 1977). In addition, the measurement must be practical so it can be accomplished as part of a field study outlined for the overall ELF ecological monitoring program. Based on these constraints, the Tree and Herbaceous Plant study has been divided into the separate elements described below. It is the objective of this study to investigate and characterize these elements on selected plots within the ELF antenna area prior to the operation of the communication system (1986). This will give baseline information on possible ELF antenna effects on the associated forest communities.

Plot Selection and Statistical Design

Due to complex interrelationships within a forest community, previous studies have not been successful in determining whether ELF fields have an effect on vegetation. McCormick et al. (1971) measured bud mortality, which has been found to be sensitive to ionizing radiation, and species diversity in a field study of ELF effects. Although each of these plant variables was considered to be sensitive parameters for ELF field influence, no conclusions could be reached due to the edge effect along the antenna right-of-way. Such a condition would cause changes in nutrient and water availability, quality and quantity of incident light, and microclimate. Greenberg (1972) also had difficulties reaching conclusions on changes in plant diversity in the ELF field due to difficulties in finding paired plots which were sufficiently alike. Similarly, in a biotron study, Gardner (et al., 1975) was able to separate ELF treatment effects from environmental effects when studying growth, stomatal resistance and transpiration.

From these studies it becomes apparent that greater control of site

environmental factors will be necessary to detect the subtle influences of the ELF electromagnetic field on the forest ecosystem. This can be partially accomplished by careful selection of treatment and control plots, taking into account all appropriate site factors that influence vegetation such as soil and microclimate. However, because of the inherent variability that will be encountered in an area as large as the ELF antenna site, exact duplication of all factors on plots exposed to ELF fields and controls would be virtually impossible to obtain. This would cast doubt on the results of any paired plot study.

A more sensitive statistical alternative to paired plots in this ELF ecosystem study is to use a variation of the completely Randomized experimental design with block (replicating). The statistical term "block" is conceptually an extension of the term "pair" (Zar, 1974). However, unlike paired plots, blocking can be used to partition and explain random variation within plant species and clones due to genotypes.

Ambient Monitoring

A forest community, both plants and animals, is a product of all site factors. The characteristics of such an ecosystem are determined by the combined influence of various environmental factors, either natural or man-induced. Any study which investigates the effects of one factor, such as an ELF electromagnetic field, on plant and animal populations must also take into account all other factors such as climatic, edaphic and physio-graphic variables. Variability in plant growth patterns within the ELF system influence area must first be related to fluctuations in microclimate before any change can be attributed to the presence of an ELF field. For example, heat summations (i.e., degree-days above some critical limit) together with soil water potential, affects the onset of tree cambial activity,

relative growth and species distribution along environmental gradients (Waring and Cleary, 1967). Integration of these environmental factors is essential when the effect of ELF field on the plant community is expected to be very subtle (National Research Council, 1977). Consequently, an adequate, statistically based ambient monitoring program established in conjunction with the various ecological studies is especially critical.

Due to the large geographic area of the proposed antenna, the localized climatic influence of Lake Superior, and the variation in topography over relatively short distances, significant differences in precipitation and temperature can be expected over the ELF antenna area for any given storm event. Weather information is available from many sources in the proposed ELF antenna area (Table 1). However, many of the stations do not adequately measure the climatic factors most critical to plant growth and no stations provide soil information. While the NOAA and FAA stations in Marquette and the Air Weather Service at KI Sawyer Air Force Base are well equipped, examination of climatic data generated by these stations shows substantial spatial variability (Table 2). This would make it impossible to accurately correlate their data with plant growth patterns and processes on the study sites. For these reasons, select microclimatic data must be gathered on each treatment site to control the inherent variability in growth processes due to environmental factors.

Tree Productivity

Tree growth is quite sensitive to environmental disturbance and can be measured in various ways. The most widely used growth measurements are diameter at breast height outside the bark (dbh) and height. Of these growth variables height is more difficult to measure, while dbh can be measured very accurately by installing permanent dendrometer bands on the stem of the tree.

Table 1. Weather Stations and Data Collected within Marquette and Dickinson
Counties.

Station	Data Collected
NOAA - Marquette	P, S, T, BP, RH, SR, W, DP
FAA - Marquette	P, S, T, BP, RH, W
KI Sawyer Air Force Base	P, S, T, BP, RH, W, DP
City of Marquette	P, S, T, RH
Ishpeming	P, T (non-recording)
Gwinn	P
Crystal Falls	P, T
Champion	P, T (non-recording)
Rock	P (non-recording)

P - precipitation

S - snowfall

T - temperature

BP - barometric pressure

RH - relative humidity

SR - solar radiation

W - wind speed and direction

DP - dew point

Table 2. Range in climatic data within Marquette and Dickinson Counties
(1940-1969)*.

Datum	Range
Annual Pressure Precipitation (mm)	762-838
Annual Average Snowfall (mm)	1778-3556
Annual Mean Number of days with 2.5 mm or more precipitation	65-85
Mean Date of First 152 mm snow depth	Nov. 11-20 to Dec. 11-20
Annual Mean Temperature (°C)	4.4 to 5.5
Annual Average Daily Minimum Temperature (°C)	0.5 to -2.2
Annual Average Daily Maximum Temperature (°C)	10 to 11.6
Average Length of 0°C Freeze-free Days	60 to 150
Average Number of Days with 152 mm of Snow on the ground per season	70 to 110

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*Data obtained from isothermic maps, Climate of Michigan, Michigan
Department of Agriculture.

Another advantage of using dbh is the responsiveness of cambial activity to environmental effects (Smith, 1962) and it's strong correlation with total biomass of the tree (Crow, 1978). Therefore, if changes in diameter growth are evident due to treatment, the magnitude and direction of the change can be quantified on a tree or stand basis. Consequently, dbh measurements are most often used in environmental impact studies.

While these measurements can provide present stand production information and a means to predict future productivity, the capacity of a stand to continue producing can be determined by monitoring reproduction and mortality. Environmental change can often affect a forest by killing existing trees or decreasing or increasing the amount of reproduction coming into a stand. Thus, to arrive at a complete picture of site disturbance on tree production, reproduction, mortality, dbh and height must all be measured.

Tree Phenology

Phenology is the science concerned with periodic biological events in plants as they are related to environmental (usually climatic) variables. For example, bud opening and shoot expansion have been found to be related to temperature, and its importance is shown by the variability among years of tree growth initiation (Kozlowski, 1971).

The timing of occurrences (often referred to as phenophases), such as initial or full flowering, leaf out, bud burst, leaf fall, etc., can also be indicators of environmental inputs (Lea, 1979). These measurements are of value in an ecological study because they are indicators of physiological processes in the trees that are being affected by environmental inputs. Therefore phenological changes would indicate physiological changes. When carefully standardized observation techniques are utilized, plants can

be considered to integrate and express the net effect of environmental variables (Lieth et al., 1971; US/IBD Phenology Committee, 1972).

Because year to year climatic variation is likely to differentially affect sites within the ELF influence zone, a preconstruction phenological and ambient data base will be essential to establish relationships between phenophases and the environment. Only if this relationship is established can potential effects of ELF be separated from climatic influences when the antenna is placed in operation. Phenological information on leaf out, senesence, and cambial activity can also be used in the analysis and interpretation of growth data by more precisely defining the growing season.

Herbaceous Plant Growth

Since composition of herbaceous plant cover has been commonly found to be influenced by environmental changes, a primary measure of response is species diversity. This can be done by using species presence alone and/or by weighing presence by coverage density and biomass (Mueller-Dombois and Ellenberg, 1974).

While clipping and weighing plant material is a more accurate method of measuring diversity and productivity, it will be avoided since it may be more important to be able to use the same microplots throughout the study. An alternative is to measure lead stem and branch stem lengths, leaf dimensions, and reproductive part dimensions periodically during the growing season for a species. These can be converted to dry-weight equivalent by using regression equations to predict dry-weights using clipped material from the same species found in nearby areas outside the study plots. Methods of estimating dry weights to plant parts similar to the above have been successfully employed by others (Shafer, 1963; Stone and Crawford, 1981).

Root Growth and Mycorrhizae Development

Optimum root growth and development is essential in maintaining
forest productivity. Fine root system (less than one millmeter
in diameter)is primarily responsible for supplying the tree with
water and nutrients. These fine roots may comprise less than 50 percent
of total root weight, but they represent more than 75 percent of total root
length (Lyr and Hoffman, 1967).

Identification and quantification of the actively absorbing portions
of root systems is ecologically important in an environmental impact study
(Bohm, 1979), and is facilitated in many tree species by the development
of characteristic ectomycorrhizae. This symbiotic plant root/fungal
relationship greatly increases root surface area, leading to greatly en-
hanced nutrient and water absorption (Gerdemann, 1974). Under natural
conditions mycorrhizal infections are prerequisite to satisfactory growth
for nearly all tree species (Vozzo, 1971; Gerdemann, 1974).

In addition to mycorrhizae being essential for tree growth, they are
also very sensitive to environmental change (Sutton, 1969; Slankis, 1974,
Mosse et al., 1981). The delicately balanced physiological relationship
which mycorrhizae represent should be monitored for possible effects of
ELF radiation since these soil/tree root organisms will be so closely
coupled with the electric field (Anonymous, 1977). Though root systems
are generally considered difficult to study, several features of ectomycor-
rhizae facilitate such investigations. Ectomycorrhizal fungi alter fine
root morphology to characteristic rootlet morphology types (Trappe, 1967;
Harley, 1969; Zak, 1971; Alvarez and Cobb, 1977), thus permitting
detection of environment-related shifts in mycorrhizal fungus populations.
In additon, the concentration of mycorrhizae near the soil surface (Lyr
and Hoffman, 1967), and the perennial nature of mycorrhizal fine roots

(Sutton, 1969) allow a simplified and statistically rigorous sampling scheme to be used.

Litter Production

As discussed earlier, trees exert a stong influence on other organisms in a forest ecosystem. One way that has been commonly used to link tree productivity and influence on the site has been through the measurement of litter production (Gosz et al., 1972). Litter production is important in the transfer of nutrients and energy within a vegetative community (Crow, 1974) and has been found to be dependent on growth processes and environmental conditions (Bray and Gorham, 1964). Because of its central role in nutrient cycling and the ease with which it is measured, litter production has been an important consideration in many ecological studies.

The production of tree litter has been found to be sensitive to many environmental conditions, such as elevation changes, soil characteristics, slope, and soil water content; as well as minor fluctuation in weather from year to year (Gosz et al., 1972). This sensitivity to site factors has also been a prime reason for using litter production as a criterion of disturbance on sites (Woodwell and Marples, 1968; Crow, 1974). Of particular interest is the success with which litter production measurements were used as an indicator of radio-sensitivity of forests. Woodwell and Marples (1968) found leaf litter decreased at Brookhaven after irradiation with gamma radiation from a Cs^{137} source. They also used litter to differentiate the relative sensitivity of tree and understory species to irradiation, as leaf production was directly related to changes in tree vigor.

Since litter samples can be gathered easily at frequent intervals, they not only would provide an estimate of forest production, but also give an insight on the time of tree component loss, such as leaves, bud scales, seeds,wood material and flower parts (Ovington, 1963). This measurement would also serve to verify on a stand basis many of the phenological

monitoring measurements that will be taken on individual trees.

Additionally, leaf samples can be taken for nutrient analysis before fall coloration to monitor the nutrient translocation from the foliage to the brances prior to leaf fall. The physiological change is also sensitive to environmental stress and would be a potential indicator of ELF field effects. Nutrient analysis of litter components collected can also be used to evaluate nutrient returns to the soil and subsequent effects on soil organisms.

METHODS

Plot Selection

Since the landscapes, forest types and sites in the proposed area are varied and complex, the careful choice of study plots will be essential to the success of the research program. The choice of these areas will be guided by existing Michigan Department of Natural Resources CFI plot data on file at Michigan Technological University.

Preliminary information gained from the CFI data base has shown that "upland aspen" is the most common forest type within the proposed ELF antenna system, comprising 34% of the area. Consequently, this cover type would give the greatest opportunity for finding suitable ELF treatment sites and controls. The species composition of the upland aspen forest (Table 3) provides a diverse mixture of fast growing and slow growing trees from which to analyze ELF field effects. An estimated 156 CFI plots located within the proposed grid area have been identified as being the aspen cover type.

Table 3. Species composition of the CFI plots within the Aspen Cover Type on the proposed ELF system area.

Trembling Aspen	49%
Bigtooth Aspen	12%
Paper Birch	10%
Balsam Fir	7%
Balsam Poplar	5%
Sugar Maple	3%
Red Maple	3%
Other (< 2%)	11%

A more detailed field examination of the existing CFI plot data will yield information on tree age, species composition, growth, mortality and disease and insect damage. Field inspection will verify this information and provide detailed information on soil morphology, slope, aspect, vegetative habitat type (Coffman et al., 1980) and occurrence and damage of specific disease and insect pests.

Selection Criteria

Once the antenna right-of-ways have been located, six ELF field influenced plots in the aspen cover type will be chosen as shown in Figure 1. Three plots will be established at each treatment site on similar soil and landform position. This change in plot design from the paired plot approach provides for improved cost efficiencies by minimizing travel and access time, reducing variability among sampling areas and decreasing the amount of equipment required to control microenvironmental variability.

Three plots will be clustered along the aboveground portion of the antenna and positioned parallel to the right-of-way to provide an "edge" effect from the right-of-way clearing. The nominal electromagnetic field strengths in excess of 0.07 volts per meter (electric) and 0.03 Gauss (magnetic) will be assured by this location. Three plots will also be clustered near the end of the below ground portion of the antenna. The plots will be positioned adjacent to the right-of-way to insure that the nominal electromagnetic field strength of 1.5 volts per meter (electric) and <0.03 Gauss (magnetic) are achieved. Three control plots will be clustered in an area exhibiting similar vegetative, stand, soils and topographic conditions as those along the ELF antenna. One of the control plots will be located on an existing CFI plot in order to utilize its growth data base. The control plot cluster will have electric fields 2

orders of magnitude less, at the soil surface, than the ELF influenced plots. A cleared strip will also be made adjacent to the control plots in order to simulate the right-of-way clearing and provide a control on the "edge" effect.

Inclusion of the "edge" effect in this design was necessitated by the fact that lateral extent of the ELF field from the underground antenna is approximately 12 m. Any plots located with an effective zone of 8 m, accounting for the right-of-way clearing, will invariably be subjected to the edge effect. Accordingly, all treatments are designed to include an edge effect in order to facilitate between treatment evaluations.

Control sites will be tentatively selected based on data analysis, field examinations of the CFI data base and the probable antenna location. Subsequently, treatment plots will be located along the antenna corridor conforming to similarities with the control site.

Ambient Monitoring

One remote ambient monitoring system will be installed in each set of treatment plots. The environmental factors to be measured and the locations are as follows:

Atmospheric:

1. Precipitation: amount, pH, nutrient content of rainfall
   and snow; depth of snowpack for each set of treat-
   ment plots (i.e. control, aboveground, belowground)

2. Relative humidity on each plot

3. Temperature: at 1 meter above ground level on each plot

4. Solar radiation for each set of treatment plots

Soil:

1. Temperature at three soil depths on each plot: litter layer
   (humus) - mineral soil interface and 5, 20 cm
   depths in the mineral soil.

2. Moisture content at three soil depths on each plot

   (same depths as temperature).

A Handar 540A Multiple Data Acquisition System will be used to provide continuous climatic and soil data logging and telemetry communications from the sites. Telemetry communications will permit complete unattended operation of the system, thereby greatly reducing costs by eliminating visits to the site to physically obtain data. Each ambient monitoring instrument package will be independently powered by a solar cell/storage battery system and will operate unattended for up to 30 days. The system will utilize the NOAA National Earth Satellite Service (NESS) to retrieve data, which is a no-cost service. Data will be retrieved on a weekly basis from NESS. Data sets will consist of mean hourly values for the above mentioned parameters, on a daily basis. The complete ambient data base will be maintained on the MTU 1108 Univac computer as part of the herbs and trees ELF data base.

The Handar systems are certified by NESS and are utilized by other federal agencies. Utilization of this system will ensure accurate measurements of key ambient factors affecting growth processes.

Tree Productivity

Yearly tree productivity measurements taken on all study plots will include diameter increment, tree height increment and mortality. In conjunction with the Tree Phenology study, dendrometer bands will be installed on all trees 10 cm dbh and larger. (Trees reaching this diameter during a growing season will have a band installed on it before the start of the next season.) Diameter increment will be measured to the nearest 0.5 mm. Height measurements will be taken to the nearest 15 cm using a Spiegel Relascope. Diameter increment will be a main response variable in this study, while height, because of the difficulty of measurement, will be used

primarily in stand and individual tree characterization.

Core samples will be taken from five trees of each species greater than 10 cm in diameter at the end of the growing season following plot establishment. Diameter increments will be measured in each core to determine the past pattern of tree diameter growth among the treatment and control plots. Dendrometer banding will monitor tree diameter/climatic effects during the baseline portion of the ecological monitoring program and during the subsequent ELF antenna operation.

Tree Phenology

Species Selection - The choice of species is limited by tree composition of the prevalent forest cover type selected for the study as a whole (i.e., aspen type). However, among the trees present, several have particular advantages for phenological comparisons. Trembling aspen is most responsive to initial favorable growth conditions in the spring and it's distinct leaf-development stages facilitates accurate recording of phenophases (Ahlgren, 1957; Kotar, 1967). Similarly, among the conifers balsam fir displays the most recognizable shoot and needle development stages. In most other coniferous species this is a less distinct, continuous process (Kotar, 1967). Based on such considerations, the following species will be used in this study: trembling aspen, bigtooth aspen, sugar maple, paper birch, and balsam fir. Five individuals of each species on each plot will be marked and numbered. All individuals of a given species will be within the same 4 cm diameter class.

Observation Techniques - For most meaningful ecological interpretation, the phenophases chosen should meet two basic requirements: 1) they must represent the entire growing season; and 2) they must be easy to observe (Hopp et al. 1969). The following phenophases will be included in this study: bud burst, leafout, flowering, fruiting, leaf coloration, leaf-

fall and cambial activity. For each of these phenomena the initial, average and terminal date of occurrence will be recorded.

The US/IBP Phenology Committee (1972) recommends that due to the subjective nature of these observations, precise descriptions of phenophases should be made for each study. In our study these criteria will be developed during the first 16 months of observation. Complete photographic record and specific developmental stages will be established and will serve as standards. This will insure year to year accuracy, especially if different observers will be involved. Cambial activity will be monitored by permanently installed dendrometer bands (devices to measure tree circumference) and will be relatively free of personal bias. All visual observations will be carried out continuously from beginning to the end of the growing season, but due to large distances between study plots, each plot will be visited every two to three days.

Herbaceous Plant Growth

Evaluation of CFI plot data indicates that three primary vegetative habitat types, Tsuga-Maianthemum-Vaccinium, Tsuga-Maianthemum, and Acer-Tsuga-Dryopteris, predominate the proposed ELF influence area (Coffman et al., 1980). The following species within these habitat types will be considered in this study:

Maianthemum canadense	Osmorhiza claytoni
Dryopteris spinulosa	Viola pubescens
Streptopus roseus	Viola canadensis
Clintonia borealis	Arisaema tryphyllum
Polygonatum pubescens	Aralia nudicaulis

This species list may be changed following preliminary reconnaissance of actual study sites. On each study plot, a minimum of four 2m x 0.5m

microplots will be established by marking the corners with metal stakes. Additional plots may be needed where herbaceous cover is relatively sparce.

Seasonal patterns of the following phenological events will be monitored and recorded by date of occurrence: emergence of vegetation growth, initiation of flowering, fruit formation, seed dispersion and senescence of vegetation parts. Periodic annual growth will be determined from selected individuals through measurements including length of main stem and branches, and leaf, flower and fruit dimensions. Due to vastly different morphology of these components among species, standard criteria for measurement correlation will be established. The two estimators of relative species dominance to be used are percentage of ground cover and frequency, both of which are dependent on size and number of individuals.

Average dry weight by species at the time of peak development will be estimated by clipping material on microplots established adjacent to the study areas. Annual trends in species composition and diversity will be monitored by calculating Jaccard's species diversity indices using presence, coverage, frequency, and biomass (Mueller-Dombois and Ellenberg, 1974).

Root Growth and Mycorrhizae Development

Species Selection - Because of the importance of distinguishing between support roots and actively absorbing fine roots, only exclusively ectomycorrhizal tree species will be studied. Endomycorrhizal roots are not morphologically distinctive, require extensive microscopy for characterization, and their fungus component can not be cultured on known synthetic media (Gerdemann, 1971). Trembling aspen, the most abundant upland species in the ELF antenna area, is partially endomycorrhizal. The most abundant exclusively ectomycorrhizal tree species are balsam fir and paper birch, comprising approximately 7 and 10 percent, respectively, of the merchantable stems in the

upland aspen cover type (Table 3). While little is known of the comparative rooting habits of tree species, it has been reported that another Betula sp., yellow birch (Bilan, 1971), and another Abies sp., grand fir (Leaphart, 1958), develop relative "intensive" root systems. Intensive rooting habit simplifies the task of collecting statistically useful samples of fine roots.

Tree species vary widely in their numbers of fungal associates. As many as 2,000 fungus species may form Douglas-fir mycorrhizae (Trappe, 1977), but only two species have been reported for paper birch and balsam fir (Trappe, 1962). A low number of fungal associates indicates less study of the tree species, but likely also reflects a relatively small number of mycorrhizal fungal symbionts. A simple ectomycorrhizal fungus population is a further reason for studying paper birch and balsam fir.

Mycorrhizae Sampling - Root weight is the most commonly used parameter of root growth response to environmental change (Bohm, 1979). Numbers of mycorrhizal and non-mycorrhizal fine roots have also been used to characterize mycorrhizal infection of yellow birch (Mason, 1975). For this study the entire root systems of naturally-occurring understory birch and fir seedlings will be carefully excavated on adjacent plot areas and destructively sampled. Non-destructive sampling techniques have disadvantages which render them unsuitable for effective monitoring of environmental influences in the field (Sutton, 1969). Initially, five seedlings on each of nine study sites will be sampled annually in the fall. Sample size will subsequently be adjusted in accord with estimated population variability.

Total weight and number of mycorrhizal and non-mycorrhizal short roots on each seedling will be documented by rootlet morphology type and expressed in comparison to total root or seedling weight. Distinct rootlet morphology types will be further characterized by isolation of mycorrhizal

fungi on Hagem's medium (Zak, 1971). Populations of mycorrhizae on individual root systems will be characterized by 1) richness (the number of different morphology types present, 2) density (the total weight of mycorrhizal fine roots of each type per unit total root or seedling weight), and 3) evenness (the measure of equality of abundances in a community) as defined by Williams (1977). These parameters provide a model-free characterization of seedling mycorrhizal populations. In this way, root condition and mycorrhizal composition will be quantified on a whole-seedling basis.

Litter Production

Litter traps will also be used to monitor tree productivity on all sites. One square meter litter traps will be constructed from wood and fiber glass screening. Four traps will be placed on each permanent measuring plot. Samples will be collected monthly during snow free periods and composited by plot. Foliar samples will be collected from lower, middle and upper crown positions of major species on each plot before leaf coloration at the end of the growing season (early September). This sampling time and crown positions have been found to be a good predictor for mineral nutrition of the tree (Lea, 1979). Nutrient data will also provide an insight on mineral translocation before leaf fall.

All samples taken back to the lab will be dried to a constant temperature at 60°C and weighed. Litter will be separated by species of leaf, seeds, twigs and other plant components. Foliar samples will be kept separated by species. Subsamples of each category of litter and all foliar samples will be ground and analyzed for N, P, K, Ca, and Mg.

Tree Disease and Insect Monitoring

It is unreasonable to expect that study sites can be located in the ELF antenna area which have all trees 1) initially devoid of diseases

and insect pests, or which 2) would remain free of pests over the period of the study. Pathogens and insects are integral components of all terrestrial ecosystems and strongly affect community dynamics (Manion, 1981). Forest pests may attack study trees directly or influence their growth by attacking neighboring trees. Obviously, the healthiest sites possible must be selected and the distribution of disease and insect pests must be monitored as an aid to overall growth data interpretation for each tree and site. In addition, the incidence of fungal and insect attack on trees growing in the ELF exposure area may be altered once the antenna is placed in operation.

Each potential treatment and control plot will be examined by a plant pathologist and forest entomologist for suitability of the plots and individual trees for use in the study. They will also perform an annual examination of the selected study trees and sites. A pest record will be kept for each tree and plot. Incidence of heartrots and root diseases will be evaluated on the basis of 1) presence of decay fungus fruiting structures (conks, mushrooms) and decay (Anderson and Schipper, 1978), and 2) tree crown condition (Parmeter et al., 1976). Occurrence of Hypoxylon and Nectria cankers will be documented on the basis of canker sizes and numbers and tree crown condition. Incidence of defoliating insects will be recorded with the severity of infestation. Wood-boring beetle infestation will be documented by insect species and quantified. These data will be available to investigators studying tree phenology, growth, and productivity.

Electromagnetic Field Strength Monitoring

Measurement of soil conductivity is critical in the selection of treatment plots and controls to insure that they meet the electromagnetic field

intensities required for this study. Seasonal variations in moisture and temperature have a pronounced affect on the conductivity of electric field through the soil and must be considered when monitoring the ecological effects of ELF fields (National Research Council, 1977). Consequently, soil conductivity measurements will be taken at time of plot selection and at monthly intervals throughout the year by Illinois Institute of Technology Research Institute or its subcontractor. Measurements for possible interference from power lines on other electromagnetic sources will also be made when treatment plots are selected.

Statistical Design

A nested completely randomized design will be used in this study to avoid the inherent problems of using paired plots in an extremely variable forest ecosystem. Although the initial objective of the study is to provide baseline information, provisions in the completely randomized design will allow the optimal use of this information to evaluate the potential influence of the ELF antenna field on plant growth. Thus the overall design of the plots (Figure 1) and the ecological experiments have been developed for this purpose.

The three treatments that will be evaluated are:

1. buried ELF field

2. overhead ELF field

3. control, no ELF influence.

In order to account for random effects due to site differences, three blocks (replications) for each treatment will be randomly established. Blocking (replicating) is a necessary statistical tool when measuring response variables on an experimental plot in field experiments. This tool is used to partition and explain random variation that is found between sites in spite

of similarities in stand composition, soils and topography (Zar, 1974).
Blocking is also necessary to eliminate the inherent variation within species,
particularly among aspen clones.

Analysis of variance and covariance will be used to evaluate the dif-
ferences found among treatments in such measured response variables as:
phenological events, diameter increment, or litter production. Analysis
of covariance will be used to adjust the treatment means for the effects
of ambient factors and stand characteristics that could randomly vary,
such as temperature, precipitation or stand density. This would result in
a better estimate of treatment (ELF field) influence on any measured
response variable and would contribute toward lowering the experimental
error. The analysis of variance and covariance techniques to be used to
analyze the various response variables are found in Table 4.

Table 4. Analysis of Variance and Covariance table with the F test for
significance.

Source of Variation	df	SS	MS	F[1]
Covariates (ambient variables)	# of variables	SS_{cov}	MS_{cov}	$\dfrac{MScov}{MSe}$
Total	48[2]	SS_{TOT}		
Between all blocks	8	SS_B		
Treatments	2	SS_{TR}	MS_{TR}	$MS_{TR}/MS_{TR(B)}$
Among blocks within treatments	6	$SS_{TR(B)}$	$MS_{TR(B)}$	$MS_{TR(B)}/MSe$
Error	40[2]	SSe	MSe	

1/The F statistic will be used to test significant differences between the
System Area and the Control Area.

2/Adjusted for covariates and species composition.

DESCRIPTION OF PROGRESS

Plot Selection

The varied and complex nature of the landscapes and forest types in the ELF antenna area requires careful selection of treatment plots and controls. Consequently, the major activity during this period was the four month screening of potential study plots. Unfortunately, much of this work had to be accomplished prior to the final selection of the ELF antenna path.

Preliminary investigation was guided by information contained in the Michigan DNR Operations Inventory and Continuous Forest Inventory (CFI) systems. CFI information showed "upland aspen" to be the most common forest type within the proposed ELF antenna zone and thus was tentatively selected for location of the study plots. The extent of the aspen would increase the likelihood of the antenna passing through this type and thus allow preliminary studies to begin prior to antenna right-of-way selection.

Approximately 150 CFI plots within the ELF antenna grid were identified as being of the aspen-type. Several CFI plots were visited and were found not to meet the study requirements. It became evident that CFI information alone would not provide the necessary data to find and evaluate potential study sites. Data from the DNR Operations Inventory System was then obtained in hopes that this information, coupled with that of the CFI system, would further broaden our information base for preliminary site selection. Forest compartments with major tree species of aspen, birch, and balsam fir were delineated and transferred to cover type maps. The CFI plots within these compartments were identified and visited in the field. The result showed 31 CFI plots occurring within the specified forest compartments located throughout the 230 square mile proposed antenna area. When visited many of these stands were found to have been recently cut and thus, were undesirable for the purpose of this research. The small number of identified stands spread throughout such a large area made preliminary site selection a cumbersome task.

When the location of the one half mile wide antenna corridor was announced in late September, plot selection efforts were concentrated in a much smaller area. Since the six ground antenna areas are relatively small, they represented the most critical areas for finding suitable sites. However, none of our previously identified stands from the CFI and Operations Inventory are located within a 1 mile radius of any of the six endpoints of the proposed antenna. A detailed field reconnaissance of the underground areas was then undertaken to locate stands that would be suitable for study. This included an aerial survey of the antenna endpoints to help direct the field crews to areas offering the best potential. These areas were then visited and the following preliminary information recorded:

* Tree species composition

* Stand basal area

* Site index

* Tree diameter distribution

* Ground flora inventory

* Soil horizon thickness

* Soil texture

* Rock abundance

* Litter composition

* Incidence of insect and disease damage

* Presence or absence of earthworms

It was found that each endpoint possessed an uniquely different forest type from the others. Topography and soil also varied tremendously from endpoint to endpoint. Within the 1 mile radius of each endpoint a mosaic of forest types makes evaluation of the area difficult, since the terminal ground antenna could be located anywhere within a three square mile area. Final site selection will depend on the exact placement of the ground antenna. At this point, aspen

stands as described in the proposal, and large enough to be used for the establishment of study plots, have not been located near the proposed endpoints of the antenna. We will continue to search these areas for stands of desired species composition but are also evaluating other forest types (and thus different species) that would enable us to monitor ELF fields in the same manner as the species within the aspen type. These types may include stands dominated by paper birch, red oak, balsam fir; mixed hardwood, paper birch, balsam fir; and aspen, jack pine. These types of stands are generally available throughout the entire antenna area. Furthermore, studies involving phenology of herbaceous plants and litter decomposition can also be easily accommodated in these other forest types. Since careful site selection is critical in evaluating ELF fields on vegetation, forest types other than the aspen type may provide a better opportunity to locate study sites within narrower environmental constraints.

Ambient Monitoring

Contact was made with engineers from Handar Inc., Sunnyvale, California, on the design and configuration of the ambient monitoring system. The proposed system specifications provide for three data acquisition systems, one for each set of treatment plots. A total of 24 sensors are to provide data inputs to each of the data acquisition systems. Testing by Handar Inc. to evaluate the system capacities to accommodate the required number of sensors and data acquisition programming has begun and will continue into the next study year.

The National Earth Satellite Service (NESS) was contacted to obtain the necessary permits to utilize the Geostationary Operational Environmental Satellite and the NESS earth station. Once the ambient system is finalized application materials will be presented to NESS. Planning was also begun to facilitate the inter-computer communications between NESS and the MTU computer system.

Tree Productivity

Construction of dendrometer bands used for measurement of tree cambial activity was started in late summer 1982. All materials for production of the bands have been ordered and received. The template used to imprint measurement increments on the bands has also been completed.

Root Growth and Mycorrhizae Development

Even though the field plots were not established during this four month period, fruiting bodies of presumptive mycorrhizal fungi were collected from birch, balsam fir, and red and jack pine stands in the ELF antenna grid area. The fruiting bodies were characterized macro- and microscopically and isolation was attempted from fresh specimens onto Hagem agar. Spore prints were made from each collection and specimens have been oven - and freeze-dried for storage. Reference specimens and cultures obtained in this manner will be used to identify mycorrhizal fungi isolated from tree roots when the ELF study sites are established.

Collections of 65 presumptively mycorrhizal fungi have been made; 17 of these have been identified and approximately 23 have been successfully isolated into culture. Genera represented in culture include Amanita, Boletus, Cortinarius, Lactarius, Leccinum and Suillus. A number of species remain to be identified to genus as well as species.

Litter Production

Construction of 36 one by one meter litter traps was complete and traps are being stored at the Ford Forestry Center until placement on the study sites next summer.

SUMMARY

The major activity during the initial four month study period was the screening of forest stands for use as ELF study or control plots. Unfortunately, much of

this work had to be accomplished prior to final selection of the ELF antenna
site. Reconnaissance of ELF grid area showed a wide mosaic of forest conditions,
topography, and soil types. Aspen stands as described in the proposal, and large
enough to be used for the establishment of study plots, have not been located
near the proposed endpoints of the antenna. A search for aspen stands of desired
species composition will continue, but other forest types will also be examined.
These types may include stands dominated by paper birch, red oak, balsam fir,
mixed hardwood, and jack pine. These stands are generally available throughout
the entire antenna area, and would be suitable for the phenology studies.
Final study site selection will not be possible until antenna placement is
known, particularly the location of the ground terminals.

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GLOSSARY

Ambient monitoring	Recording of existing climatic factors such as temperature, wind speed, precipitation, soil temperature and moisture, and solar radiation.
Basal area	The area of the cross section of a tree at DBH.
Biomass	The amount of living matter in a unit area.
Combial activity	The wood building process of the tree cambium which results in increased diameter.
CFI	Continuous Forest Inventory - a system of permanent growth plots which are measured periodically.
DBH	Diameter at breast height. Average stem diameter, outside bark, at a point 4.5 feet above the ground.
Dendrometer band	A permanent devise placed on a tree for measuring diameter growth.
Ectomycorrhizae	A type of mycorrhizae in which the fungi grow only intercellularly and produce an external mantle.
Edge effect	Changes in environmental conditions near the forest edge due to natural openings or timber removal.
Endomycorrhizae	A type of mycorrhizae in which the fungi penetrate the host root cells, but do not produce a mantle.
Habitat type	Land areas potentially capable of producing similar plant communities at maturity.
Herbaceous plant	A plant that does not produce persistant woody tissue.
Litter	Dead, unincorporated leaves, twigs, seeds, plant parts, etc. on the forest floor.
Mycorrhizae	An association between plant root tissues and fungal mycelia.
NESS	National Earth Satellite Service
NOAA	National Oceanographic and Atmospheric Administration
Phenology	The science concerned with periodic biological events in plants as related to environmental variables.
Phenophase	The timing of phenological events.
Species diversity	The number of different species and the amount of each in a given area.

Figure 1

PROJECTED FIELD LAYOUT FOR ELF PROJECT
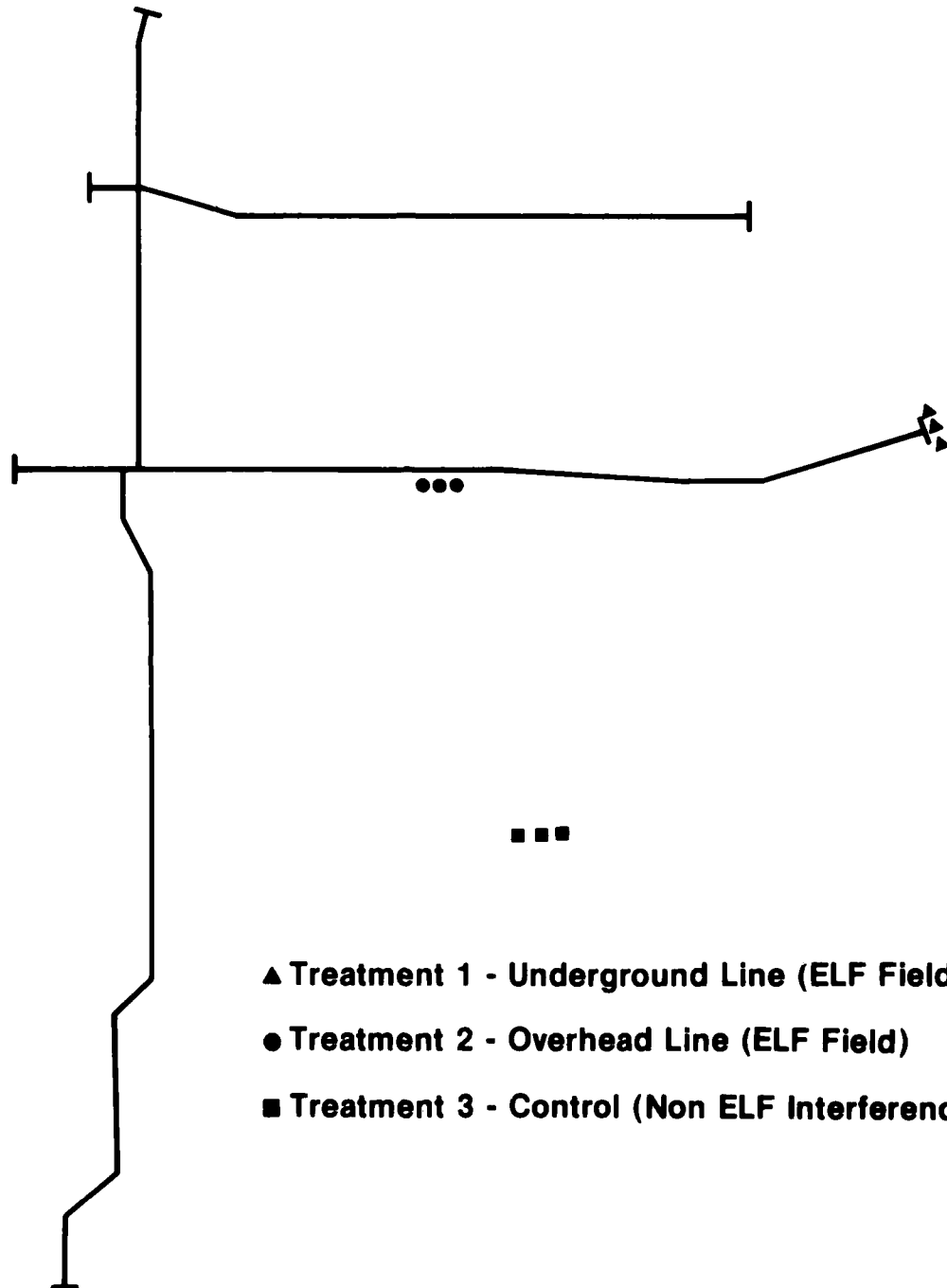
▲ Treatment 1 - Underground Line (ELF Field)

● Treatment 2 - Overhead Line (ELF Field)

■ Treatment 3 - Control (Non ELF Interference)

ELF COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM:
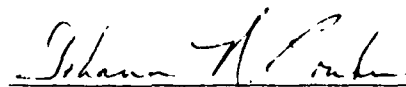
LITTER DECOMPOSITION AND MICROFLORA.

The Michigan Study Site

ANNUAL REPORT, 1982

SUBCONTRACT NUMBER: E06516-82-C-40015
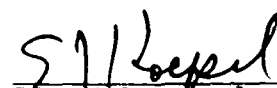
PROJECT MANAGER: Johann N. Bruhn
                        Assistant Professor

PRINCIPAL INVESTIGATORS: Susan Bagley
                            Johann Bruhn
                            Martin Jurgensen

RELEASING AUTHORITY: Edward J. Koepel
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TABLE OF CONTENTS

ABSTRACT

The litter decomposition component of a forest ecosystem comprises a complex of processes and microbial populations responsive to environmental perterbations and contributory to tree vigor. Forest litter decomposition and microflora studies are particularly appropriate to the ELF Environmental Monitoring Program because of 1) the dominance of forest vegetation on the proposed ELF communications antenna area and 2) distribution features of the induced field in the forest floor.

The overall objective of these studies is to quantify key decomposition processes and decomposer populations within the ELF antenna area prior to the actual operation of the communication system in 1986. Subsequent study will evaluate the possibility of ELF induced effects. Six work elements have been defined for the purpose of achieving the study objective: 1) plot selection, 2) ambient monitoring, 3) litter decomposition/nutrient flux, 4) nitrogen cycling, 5) non-mycorrhizal rhizoplane fungi, and 6) rhizoplane actinomycetes.

Activity during the initial one month period centered on evaluation of potential study sites, establishment of a pilot litter decomposition experiment and establishment of protocol for microflora samples. Final selection of study sites and study tree species necessarily awaits firm location of the antenna course.

INTRODUCTION

The litter decomposition subsystem of any forest ecosystem serves to 1)transform the essential plant nutrients in organic matter into forms available for root uptake, 2) pool the nutrients collected by primary producers, and 3) release these nutrients in a regulated fashion for re-use by the autotrophs. The energy provided by litter decomposition also fuels heterotrophic dinitrogen fixation and capture of nutrients washed from the atmosphere or leached from living plants. Due to the large quantities of potentially available plant nutrients found in the litter component of forest biomass, knowledge of key decomposition processes and their rates is essential to conceptualization of ecosystem dynamics. Organic matter decomposition is primarily accomplished by heterotrophic microorganisms whose activities are regulated by the environment. Recognizing the delicate balance of ecosystem functioning, it is apparent that environmental factors which disrupt decomposition processes detract from the optimum flow of nutrients to vegetation.

As one such environmental factor, ELF electromagnetic fields merit investigation for possible effects on the litter decomposition subsystem. This study is designed to 1) quantify and characterize the populations of major rhizoplane fungi and actinomycetes and 2) quantify the integrated activities of microflora by measuring rates of ammonificiation, nitrification, heterotrophic dinitrogen fixation, litter mass loss, and litter nutrient (N, P, K, Ca, MS, and S) flux. (While microfaunal populations are being characterized by other parties, the integrated influences of all soil/litter organisms are reflected in these process measurements.)

Plot Selection and Statistical Design

Litter Decomposition and Microflora studies will be conducted at the same sites established for ambient monitoring and study of forest vegetation, sharing the same underlying statistical basis.

From previous studies of ELF fields, it is apparent that greater control of site environmental factors is necessary if subtle influences of ELF electromagnetic fields on the forest ecosystem are to be detected. This can be partially accomplished by careful selection of treatment and control plots, taking into account all appropriate site factors that influence vegetation such as soil and microclimate. However, because of the inherent variability that will be encountered in an area as large as the ELF antenna site, exact duplication of all factors on plots exposed to ELF fields and controls would be virtually impossible. This would cast doubt on the results of any paired plot study.

Significant differences in precipitation and temperature can be expected among plots for any given storm event. By monitoring these and other random parameters on each site rather than one central location and using them as covariates in the statistical analysis of response variables (i.e., decomposer populations, process rates) better estimates of possible ELF treatment effects will be obtained.

A more sensitive statistical alternative to paired plots in this ELF/ ecosystem study is to use a variation of the completely randomized experimental design with blocking (replication). The statistical term "block" is conceptually an extension of the term "pair" (Zar 1974). However, unlike paired plots, blocking can be used to partition and explain random variation that is often found between plots even when the sites are carefully chosen to be similar in vegetation, soils, and microclimate. Blocking is also necessary to eliminate the inherent variation within plant species due to genotypes.

The measurement of the selected parameters to gain environmental baseline information prior to antenna use will be greatly enhanced by using existing Continuous Forest Inventory (CFI) plots in the proposed study area. CFI is a specialized forest inventory system relying on permanently located sample plots for measurement of forest change and growth (Meteer 1965). These plots give detailed information on tree growth rates and responses to past environmental inputs. Over 1,000 CFI plots have been installed in the proposed ELF communications antenna area on the Copper Country and Escanaba River State Forests by the Michigan Department of Natural Resources and monitored by Michigan Technological University research personnel. This study has provided 28 years of information on tree growth, reproduction, mortality and disease incidence as it relates to environmental influences. These results coupled with an ongoing environmental monitoring program provide a degree of accuracy seldom available in an ecosystem study of this nature. Although it is not known whether the antenna influence zone will contain CFI plots, the treatment controls may be located at these sites.

Ambient Monitoring

The ambient monitoring program funded in conjunction with the forest vegetation studies will serve admirably for decomposition studies. The sole modification involves installation of an additional set of soil temperature and moisture probes at the critical organic/mineral soil interface to more accurately characterize variation in these parameters.

Sensitivity of tree root/mycorrhizae studies is enhanced through use of the rhizoplane as the sampling universe for decomposer populations. Mycorrhizae are known to interact functionally with rhizoplane fungi and actinomycetes (Knutson et al. 1980, Marx 1982). Knowledge of rhizoplane microbial populations increases the likelihood of detecting root-associated ELF effects. Study of the rhizoplane also ties decomposition studies back to the dominant vegetation.

Finally, due to the shallow occurrence of a major proportion of feeder roots, sampling from the organic horizons ties in closely with the rest of the decomposition-related work proposed here.

Litter Decomposition/Nutrient Flux

Overall litter mass loss has traditionally been used as a measure of fully integrated litter decomposition (Kendrick 1959, Jensen 1974, Millar 1974). It has been shown, however, that the accuracy of mass loss as a sensitive index of organic matter deterioration declines with time beyond approximately one year, while nutrient flux provides continuously meaningful ecological information (Start 1972, Graustein et al. 1977, Bruhn et al. 1979, Knutson et al. 1980, Bruhn 1981). Microfloral population shifts have been shown to influence the rate of overall litter decomposition (Mitchell and Millar 1978). Effects of heavy metal inputs on litter decomposition have also been studied (Siccama 1978, Siccama et al. 1980).

Study of litter decomposition greatly extends the usefulness of litter productivity data collected in the course of forest vegetation studies. Knowledge of litter biomass production and nutrient content serves as the basis for decomposition study. Further, the method of study integrates the activities of microflora with those of all but the largest artropods and earthworms, extending the value of all population data.

Nitrogen Cycling

Rates of litter breakdown by the soil microbiota are strongly affected by the status and cycling of nitrogen in the organic materials (Kelly and Henderson 1978, VanLear 1980). The release of mineral nitrogen from organic matter is commonly measured as a rate function of litter decomposition (Keeney 1980). Nitrogen is unique among plant nutrients because of its presence almost entirely in organic forms. No inorganic reserve is normally present

to alleviate nitrogen losses to volatilization or leaching (Wollam and Davey 1975).

In natural ecosystems, atmospheric N_2 is converted into forms useful to plants through dinitrogen fixation. In order to become available for plant uptake, resulting organic nitrogen complexes must first undergo transformation to ammonium (ammonification) by the soil microflora. In many ecosystems, such as the aspen-mixed conifer cover type found in the proposed ELF system study area, ammonium is rapidly converted to nitrate (nitrification) by a relatively select group of soil bacteria (Vitousek and Melilla 1979).

The diverse group of soil organisms participating in the various segments of the nitrogen cycle are affected by the same factors which influence overall decomposition of organic matter: kind and nutrient content of organic matter; soil moisture and temperature; and populations of soil micro- and macro- fauna and flora (Witkamp and van der Drift 1961, Bartholomew 1965). These same soil organisms have also been found to be extremely sensitive to environmental perturbations such as timber harvesting, air pollution, acid rain, and heavy metal additions (Jurgensen 1979, Matson and Vitousek 1981). Since different segments of the microflora are active in dinitrogen fixation, ammonification, and nitrification, rates of these nitrogen transformations are excellent indicators of soil microorganisms' responses to ELF fields.

Rhizoplane Fungi and Actinomycetes

Litter decomposition is a complex process involving a variety of organisms engaged in the degradation of a wide range of organic compounds. The primary agents of organic matter decomposition are the fungi and bacteria. Within these broad groups, a relatively small cadre are responsible for degradation of complex structural materials such as cellulose and lignin. Among the fungi, cellulose and lignin degradation are accomplished by members of the

Basidiomycetes and Ascomycetes, including their imperfect states (Harley 1971, Jensen 1974, Mikola 1958, Millar 1974, Pugh 1974). Of the bacteria, members of the Actinomycetes have been found to degrade cellulose (Crawford 1978 Teslinova et al. 1981) and lignin/lignocellulose (Sutherland et al. 1979, Antai and Crawford 1981) in both coniferous and deciduous litter systems (Goodfellow and Dawson 1978, Kauri 1978, goodfellow and Cross 1974, Andrews and Kenerly 1979, Antai and Crawford 1981, Ogawa et al. 1981).

Population changes resulting from environmental perturbations have been documented for both microflora groups. For example, fungal and bacterial populations are sensitive to various air pollutants including acid precipitation (Hibben and Stotzky 1969, Treshow et al. 1969, Jordan and LeChevalier 1975, Manning 1976, Smith 1976, Bruhn et al. 1979, Jurgensen 1979, Bruhn 1981) and heavy metal additions (Jurgensen 1979). Changes in decomposer fungus populations have been shown to affect litter decomposition (Mitchell and Millar 1978).

METHODS

Plot Selection

Since the landscapes, forest types and sites in the proposed area are
varied and complex, careful choice of study plots is essential to
the success of the research program. The choice of these plots is being
guided by existing Michigan Department of Natural Resources CFI plot data
on file at Michigan Technological University. Preliminary information gained
from the CFI data base has shown that "upland aspen" is the most common forest
type within the proposed ELF antenna system, comprising 34% of the area.
Consequently, this cover type should give the greatest opportunity for find-
ing suitable ELF treatment and control sites. An estimated 156 CFI plots
located within the proposed grid area have been identified as being the aspen
cover type. The species composition of the upland aspen forest is presented
as Table 1.

Table 1. Species composition of the CFI plots within the Aspen Cover Type
on the proposed ELF system area.

Trembling Aspen	49%
Bigtooth Aspen	12%
Paper Birch	10%
Balsam Fir	7%
Balsam Poplar	5%
Sugar Maple	3%
Red Maple	3%
Other (≤ 2%)	11%

Three plots will be clustered along the aboveground portion of the
antenna and positioned parallel to the right-of-way to provide an "edge"
effect from the right-of-way clearing. The nominal electromagnetic field
strengths in excess of 0.07 volts per meter (electric) and 0.. : Gauss
(magnetic) will be assured by this location. Three plots will also be
clustered near the end of a below ground portion of the antenna. The
plots will be positioned adjacent to the right-of-way to insure that the

nominal electromagnetic field strength of 1.5 volts per meter (electric) and <0.03 Gauss (magnetic) are achieved.

Each plot for the below ground antenna treatment will be subdivided in order to evaluate any potential effects from the voltage gradient. Two subdivisions will be made, one encompassing 4-2.5 volts per meter field strength and the second encompassing the 2.5 to 1.5 volts per meter field strength. This design will reduce experimental field strength variability and provide for a complete analysis with near maximum field strengths.

Control site selection will be based on analysis of the CFI data base and field examination of the probable antenna location. Subsequently, treatment plots will be located along the antenna corridor conforming to similarities with the control site. One of the control plots will be located on an existing CFI plot in order to utilize its growth data base. The control plot cluster will have electric fields 2 orders of magnitude less, at the soil surface, than the ELF influenced plots.

A strip will be cleared adjacent to the control plots in order to simulate the right-of-way clearing and provide a control on the edge effect. Inclusion of the "edge" effect in this design was necessitated by the fact that lateral extension of the ELF field from the underground antenna is approximately 12 m (Personal Communication; Keith Stanek, Dept. of Electrical Engineering, MTU). Any plots located with an effective zone of 8 m, accounting for the right-of-way will invariably be subjected to the edge effect. Accordingly, all treatments are designed to include an edge effect in order to facilitate between-treatment evaluations.

Ambient Monitoring and Electromagnetic Field Strength Monitoring

The ambient monitoring and ELF field monitoring programs funded as part of the forest vegetation studies will serve decomposition studies equally well.

Statistical Design

A nested completely randomized design will be used in this study to avoid the inherent problems of using paired plots in an extremely variable forest ecosystem. Although the initial objective of the study is to provide baseline information, provisions in the completely randomized design will allow the optimal use of this information to evaluate the potential influence of the ELF antenna field on experimental variables.

The three treatments that will be evaluated are:

1. buried ELF field

2. overhead ELF field

3. control, no ELF influence

In order to account for random effects due to site differences, three blocks (replications) for each treatment will be randomly established.

Analysis of variance and covariance will be used to evaluate the differences found among treatments in measured response variables, including litter mass loss, nitrogen mineralization processes, litter nutrient content and microbial population parameters. Analysis of covariance will be used to adjust the treatment means for the effects of ambient factors and stand characteristics that could randomly vary, such as temperature, precipitation or litter nutrient content and pH. This will result in better estimation of treatment (ELF field) influences on any measured response variable and will contribute toward lowering the experimental error. The analysis of variance and covariance techniques to be used to analyze the various response variables are found in Table 2.

Table 2. Analysis of Variance and Covariance table with the F test for significance.

Source of Variation	df	SS	MS	F[1]
Covariates (ambient variables[2])	# of variables	SS_{cov}	MS_{cov}	$\dfrac{MS_{cov}}{MSe}$
Total	3/	SS_{TOT}		
Between all blocks	8	SS_B		
Treatments	2	SS_{TR}	MS_{TR}	$MS_{TR}/MS_{TR(B)}$
Among blocks within treatments	6	$SS_{TR(B)}$	$MS_{TR(B)}$	$MS_{TR(B)}/MSe$
Error	3/	SSe	MSe	

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[1] The F statistic will be used to test significant differences between the System Area and the Control Area.

[2] Include soil moisture and temperature, precipitation frequency and intensity, solar radiation, ELF.

[3] Adjusted for covariates and species composition.

Litter Decomposition/Nutrient Flux

Litter decomposition will be quantified as percent change over time in overall mass and nutrient (N, P, K, Ca, Mg, S) masses. Analysis of litter nutrient content will be conducted by the Soils Analysis Laboratory, Department of Forestry, Michigan Technological University. Fresh-fallen paper birch and red pine foliar litter will be collected from one of the control study sites annually in the autumn. Paper birch was selected for study because 1) it is one of the two initially targeted tree species for root growth/mycorrhizae and rhizoplane microflora studies, 2) mixed species litter samples would greatly increase the variation of decomposition data without contributing value to the study, and 3) use of balsam fir litter would require use of much finer mesh which could influence access to soil organisms and the microenvironment for decom-

position. Red pine was selected for preliminary study because 1) it has been included in numerous other litter decomposition and mycorrhizae studies, 2) it is easily planted for study purposes, and 3) it is native to and fairly widely distributed over the study area. Collection at one location will avoid differences which might be present between different stands on different sites. Four 15 g samples of each litter species collection will be analyzed for fresh:dry weight ratio. Initial nutrient content will be obtained from analyses performed as part of the forest vegetation litter production studies. White nylon mesh (3 mm) envelopes (15 cm x 25 cm), each containing 15 g of litter, will be placed beneath red pine or paper birch trees on each of the nine study plots before snowfall. One subset of envelopes will be retrieved after snowmelt and another subset after 12 months for analysis of change in overall and nutrient masses. The choice of 3 mm mesh for envelopes permits normal activity of the microflora as well as all but the largest arthropods and earthworms.

Rhizoplane Fungi and Actinomycetes

Fungus and actinomycete populations will be determined for feeder root rhizoplanes of birch and a conifer species occurring at or above the organic layer/mineral soil interface. Paper birch and balsam fir were initially designated as target species for two reasons. First, these are the most frequently encountered ectomycorrhizal hardwood and conifer species on the aspen CFI plots in the study area. As such, they were initially selected for root growth/mycorrhizae study. There is clear advantage to relating non-mycorrhizal rhizoplane microflora to ectomycorrhizae. Second, the variation in aspen species due to partial endomycorrhizal habit and clonal genetics is avoided. Reasons for considering red pine as an alternative conifer study species have already been mentioned in the foregoing section. Initial

sampling will coincide with fall tree root/mycorrhizae sampling. Subsequently sampling is planned to take place three times annually: immediately following snowmelt; at the point of full foliage expansion; and at the time of autumn root/mycorrhizae sampling. Insofar as possible, sampling will be conducted in the vicinity of decomposition study trees. Feeder roots washed in distilled water to remove soil particles and litter fragments will be plated onto water agar and malt extract - ortho phenyl phenol - streptomycin agar media for the purpose of isolating non-mycorrhizal fungi associated with feeder roots. The number of feeder roots to be sampled will be determined by preliminary analysis of species increment patterns for each tree species (Gochenaur and Whittingham 1967, States 1981).

Fungal populations will be characterized for comparison of 1) species richness (number of species), 2) a species list in order of decreasing abundance, 3) frequency (number of colonies per mm feeder root: overall value by tree species plus partial values for individual fungus species by tree species), and 4) model-free evenness (relative equality of species abundance). This method of characterizing decomposer populations is considered superior to commonly used diversity indices (Williams 1977). Fungi will be identified from cultures by Dr. J. N. Bruhn and/or Dr. M. J. Larsen.

Rhizoplane actinomycetes will be quantified using a dilution-plate technique. Serially diluted surface-sterilized feeder root macerate samples will be plated onto starch-casein agar containing cycloheximide and nystatin, to inhibit fungal growth (Goodfellow and Dawson 1978, Andrews and Kenerly 1979). After incubation, the total numbers of actinomycetes per rhizoplane sample will be determined. Colonies on representative plates will then be transferred (using replica plating techniques) to media containing cellulose and lignocellulose (Crawford 1978, Teslinova et al. 1981). After further incubation, the numbers of actinomycetes degrading these complex organic compounds will be recorded.

All data will be recorded as numbers of colonies per mm feeder root.

Representative actinomycete colonies from each test site (found on the original isolation media) will be selected for use in taxonomy studies. Each isolate will be restreaked onto starch-casein agar (minus antibiotics) to obtain pure cultures. Identification of the isolates, conducted by Dr. S. T. Bagley, will be based on examination of the vegetative colonies and spores, production and color of soluble pigments, melanin reaction, and utilization of various carbon sources (Williams et al. 1971, Arai and Mikami 1972, Knutsen et al. 1980).

Data related to actinomycete density, activity, and type will be analyzed in a manner similar to the data from the fungus studies, with additional calculations on species diversity (Lloyd et al. 1968). As actinomycete numbers and activities are sensitive to pH, this parameter will also be measured in the field.

Nitrogen Cycling

Nitrogen mineralization occurring during litter decomposition will be determined first during the winter of 1982/1983 (to establish techniques) and then monthly from early May to early December at the actual study sites. This sampling schedule will be used throughout the study period.

Due to the long duration of the ELF ecological monitoring program and the mixed litter composition of the sites to be monitored, the trenched plot technique (Gosz 1980) rather than litter bag incubation (Westermann and Crothers 1980) will be used to monitor the nitrogen release patterns. Six root-free plots will be established on each study plot (two in association with each litter decomposition study tree) by cutting off all roots to a depth of 30 cm (with a spade) on the perimeter of a 2 x 2 m area. Plastic will then be inserted into the spade slit to prevent root regrowth into the trenched soil. All herbaceous vegetation will be removed from the treated area.

A composite sample of three 5 cm diameter cores will be taken from the litter layer (O_1 and O_2 horizons) in each trenched area at monthly intervals (as stated earlier). Litter samples will be collected to determine oven-dry weights. In the laboratory, ammonium and nitrate determinations will be performed on the collected litter (before drying) within 24 hours after collection using automated spectrophotometric techniques. Data will be expressed as ug ammonium or nitrate per gram oven-dry litter.

Nonsymbiotic dinitrogen fixation associated with litter decomposition will be monitored on the same schedule as for nitrogen mineralization (above), with the exception that at least one sample will be collected in mid-winter (snow pack conditions permitting).

Nitrogen fixation rates will be estimated using an adaptation of the acetylene reduction technique described by Hardy et al. (1973). At each treatment plot, composite samples of the litter layer (O_1 and O_2 horizons combined) will be placed into seven gas-tight plexiglass chambers measuring 15 cm W x 15 cm L x 8 cm H. These chambers will be injected with a known volume of acetylene (final concentration of 10% acetylene: 90% air) and then placed in the litter layer for incubation at ambient soil temperature (two beneath each decomposition study tree). One of the seven replicates will not be injected with acetylene to provide a control. At the end of 12 hours, gas samples will be removed using Vacutainer vials (Becton-Dickinson Co.). These gas samples will be analyzed in the laboratory for levels of acetylene and ethylene using a gas chromatograph (McNabb and Geist 1979). Litter samples will also be collected to determine oven-dry weights. Nitrogen fixation/acetylene reduction rates will be expressed as ng N_2 fixed per gram oven-dry litter per day.

DESCRIPTION OF PROGRESS

Plot Selection

The Litter Decomposition and Microflora Study program will be conducted on
the same system of plots as the Herbaceous Plant Cover and Tree Study. In this
way, advantage can best be taken of the intensive screening for potential study
areas conducted by the Herbaceous Plant Cover and Tree Study. Coordination
between the two projects has provided field staff with insight into the overall
study site requirements. Unfortunately, final selection of study plots yet
awaits firm location of the ELF antenna course. Final selection of study tree
species as well depends on the final location of ELF antenna endpoints, due
to the small area covered by each endpoint and the heterogeneity of forest
cover types represented in the vicinities. Fortunately, several equally
satisfactory alternative hardwood and conifer species are distributed over
the area, should it prove impossible to use both paper birch and balsam fir.
Equally suitable alternative species include red oak, jack pine and red pine.

Ambient Monitoring

The Litter Decomposition and Microflora Study will share the ambient
monitoring system funded through the Herbaceous Plant Cover and Tree Study.
Coordination with ambient monitoring personnel will ensure establishment of a
system which optimizes integration of the two studies. The primary focus
of attention centers on the number and placement of soil moisture and
temperature sensors. Field work to date suggests that the organic matter/
mineral soil interface is the key depth for providing information relevant
to root growth/mycorrhizae as well as litter decomposition and microflora
studies. A second monitoring level approximately 10-15 cm lower would provide
additional useful but less critical data.

Litter Decomposition/Nutrient Flux

A preliminary litter decomposition study is being established using red

pine and paper birch foliar litter in order to test methods and characterize measures of central tendency for litter decomposition parameters. Litter envelopes constructed of white nylon mesh (3 mm) fabric will contain 15 g fresh weight of current year pine or birch litter. Materials will be placed in the field prior to final snow cover. Sample size and number, envelope construction and experiment duration for subsequent experiments will be based on the results of this pilot study.

Rhizoplane Actinomycetes

Formal protocal for processing feeder root specimens and resulting actino-mycete cultures has been developed, particularly with the view of interfacing with mycorrhizal and non-mycorrhizal fungal populations analysis. Taxonomic keys for identification of relevent actinomycete species have been acquired and a computer program for the identification process has been developed.

SUMMARY

Activity during the short life of this study to date has centered on screening of available potential study sites and tree species, establishment of a pilot litter decomposition/nutrient flux experiment and establishment of laboratory protocal for rhizoplane microflora population analysis. Final selection of study sites and tree species awaits location of the ELF antenna course, particularly the exact identification of ground terminal locations and design.

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"ELF Communications System Ecological Monitoring Program": The Effects of Exposing the Slime Mold Physarum polycephalum to Electromagnetic Fields

November, 1982

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Abstract

Laboratory exposure of the slime mold <u>Physarum polycephalum</u> to weak electromagnetic fields results in a lengthened mitotic cycle and depressed oxygen consumption. This research program has been designed to ascertain if the same physiological effects are obtained when <u>Physarum polycephalum</u> is exposed to electromagnetic fields in the vicinity of the Wisconsin Test Facility at Clam Lake, Wisconsin.

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Introduction

Extensive experiments in our laboratory have shown that applying electromagnetic fields (EMF) similar to those expected in the vicinity of the Wisconsin Test Facilities' extremely low frequency (ELF) antenna depresses the rate of respiration and lengthens the mitotic cell cycle in Physarum polycephalum (1,2). Based on these results, we now seek to determine whether similar effects are observed with both intermittent electromagnetic field exposure in the laboratory and under actual field conditions at Clam Lake. The specific questions posed are:

1. How does intermittent EMF exposure in the laboratory effect respiration and mitosis in Physarum polycephalum?

2. Does field exposure under and around the Wisconsin Test Facility antenna depress the rate of respiration in Physarum polycephalum?

3. Does field exposure under and around the antenna lengthen the mitotic cell cycle in Physarum polycephalum?

To attain these objectives, the research program has been subdivided into several smaller, more definable task elements.

Element I. Selection of control and experimental sites at the Wisconsin Test Facility.

Three primary sites were selected (2 exposure and 1 control) for experiments to be performed in the vicinity of the Wisconsin Test Facility; an alternative exposure and control site was also selected. The exposure sites are located at the antenna ground and at a point where the antenna passes under a highway. The control site is located 10 miles from the exposure sites where ambient fields are at least two orders of magnitude lower than either experimental site. A more complete description of the

sites and their attendant field levels is summarized in Appendix A.

Element 2. Development of protocols for field experiments with Physarum polycephalum.

Exposure chambers were constructed to facilitate field exposure of Physarum polycephalum while maintaining the mold in an axenic state. The exposure system includes a 10" x 10" x 12" polyethylene container with a tight fitting lid (see Fig. 1); the container will be placed 16"-20" in the ground. A vent pipe with a filter is attached to the lid of the container. Copper electrodes attached to the outer wall of the container will conduct the electric field to the chamber; leads will then be run from the electrodes to 15 cm petri plates with stainless steel electrodes attached to the top of the plate. Non-exposed "controls' and the EMF-exposed cultures will be used to determine the effects of actual field exposure on mitosis and respiration.

Elements 3 & 4. Establishment of a laboratory exposure system and determination of the effects of intermittant EMF exposure.

Prior to implementation of the field experiments in the Spring of 1983, a laboratory simulation of field conditions similar to those expected at the antenna site is being performed. To date microplasmodia currently growing in submerged shake flask cultures have been subjected to intermittent (16 hrs/day, 5 day/wk) electromagnetic fields of 60 Hz, 1.0 G, 1.0 V/m for 49 days.

Parameters compared include the rate of respiration or QO_2 (ul O_2 consumed/mg protein/min) and the length of the mitotic cell cycle. Oxygen consumption is measured using an oxygen electrode (YSI Model 53, Yellow Springs, Ohio). Microplasmodia are removed from submerged

shake-flasks, placed in the temperature controlled chamber of the instrument, and the time required to consume 20 ul of oxygen is determined. Protein estimations are performed using the Biuret procedure and protocols described previously (3)

Experiments on intermittent EMF exposure on mitosis have been under way for 30 days; too few data are available to draw any conclusions. All experiments designed to determine the length of the mitotic cycle in control and EMF-exposed cultures are scored twice. The exposure history of the cultures being scored is unknown to the technician performing the original determination of the mitotic indices. Blind scoring of mitosis is accomplished by placing both control and exposed mitoses plates in the control incubator; plates are only identified with a numerical code. The slides are rescored by E. Goodman to validate the technician's determination of the mitotic indices.

Element 5. Development of protocols for measuring O_2 consumption at the Wisconsin Test Facility.

Since cultures in the field will be maintained on a semi-solid, agar substrate, O_2 consumption will be measured by a method that is directly compatible with this form of growth rather than the oxygen electrode system which has a requirement for a liquid environment. We recently ordered an S-3A oxygen analyzer from Applied Electrochemistry to perform these analyses. Using this instrument, a disc of agar containing plasmodium is placed in the analytical cell, and the rate at which O_2 is depleted from the gas phase is determined. To normalize the data, the plasmodium is removed from the agar and the protein estimated using the Bradford procedure (4).

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Element 6. Development of computer programs for data analysis.

Computer programs are being prepared for (1) data entry, (2) screening, and (3) analysis. Several commercial programs have been purchased for tasks 1 and 2; these programs are presently being modified to tailor them for this application. Task 3 awaits completion of tasks 1 and 2.

The data entry programs will permit direct entry of raw data via a computer terminal located in the laboratory. Screen prompts will request data of a particular type (oxygen uptake or mitosis data) in a format that minimizes error. Entries that lie outside of a reasonable range will be highlighted and the technician will be asked to confirm these values. Tests for outliers will also be performed at the time of entry and outliers will be highlighted so that the technician may check the values. After these initial checks are made the data and notes regarding the experiment will be sorted in a master file for subsequent review and analysis.

It is our current plan to prepare graphical representations of data from each experiment in a format which will permit inclusion in the laboratory notebook along with the record of the raw data. This will permit rapid visual comparison of experiments and will also help identify the existence of systematic errors.

Two statistical tests will be employed to analyze the data: the paired t-test and the Wilcoxon signed ranks test (Mann-Whitney test). Both tests will be routinely used in our analyses; a difference between the results of the two tests indicates that the distributions are not normal, in which case the Wilcoxon test is the preferred test. Data collected from control and EMF exposed cultures on a given day are treated as paired groups. The average value of the control data is subtracted from each of the

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measurements taken on the experimental cultures; the data are then plotted or analyzed. The data will be displayed as histograms, if there is no difference between control and EMF-exposed cultures the histogram will be centered at 0.0. The width of the histogram will provide a measure of both the natural variability of the organism and the variability in the parameter being measured.

The precise length of the induction period is difficult to access because there is no widely accepted criteria for establishing the onset of an EMF effect. We have thus adopted the arbitrary criterion of waiting until three successive measurements (on 3 different days) reveal a difference in either the length of the mitotic cycle or respiration rate that is significant at the $P < 0.01$ level before we claim an effect.

Data obtained from cultures exposed to the same field conditions but different times will also be checked for reproducibility. Reproducibility is checked by constructing distributions for each set of cultures and then testing to determine if a significant difference between distributions exists. A Wilcoxon signed ranks statistic with $P < 0.01$ is necessary to establish a difference between distributions.

Element 7. Development of procedures to maintain _Physarum_ _polycephalum_ in the field.

The performance of field experiments on mitosis and respiration at the Wisconsin Test Facility requires that the plasmodium be maintained in an axenic state. To maintain sterility, the inside of all test containers will be washed with 70% alcohol and exposed to uv light for 12 hours prior to transfer. Plasmodia will be grown on 15 cm oatmeal-agar plates (prepared at UW-P) and transported to the test site. At each experimental site, the

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chamber will be removed from the ground and carried to the mobile lab; the lid is washed with 70% alcohol, and the petri dish removed. A small block of agar will be removed and subcultured to a fresh plate using sterile technique (a transfer hood will be located in the mobile lab). The remaining plasmodium will be used for either O_2 measurements or brought back to UW-P for cell cycle analysis.

Appendix A

Selection and Characterization of Field Sites

Five field sites in the Chequamegon National Forest have been identified, and the fields at these sites were characterized by the research team and a team from IITRI that included J. Zapatowski, J. Gauger, and J. Ertl. This work was done during a visit to the Clam Lake area on September 23-24, 1982. The sites were selected on the basis of their being generally similar in natural setting and being appropriately located with respect to the antenna. All sites are shaded by trees to prevent over heating of the cultures by direct sunlight. The single "antenna" site is located at the edge of the right-of-way near the buried portion of the main north-south antenna cable at a highway crossing. The two "ground" sites are located at the edge of the right-of-way at a point near the buried ground cable. The two "control" sites are in conveniently located parts of the forest that are similar to the others in their general vegetation, etc.

In characterizing the fields at each site, five types of measurement were made:

1. Electric field strength in two horizontal directions, parallel and perpendicular to the antenna, with the antenna energized. Separate measurements were made with eacharm of the antenna energized at 250A.

2. Magnetic field strength in three directions, parallel, perpendicular, and vertical, with the antenna energized as above.

3. Electric field source impedance in the direction of maximum field with both arms energized simultaneously at 250A.

4. Background elelctric field strength, measured as above with the

antenna off.

5. Background magnetic field strength, measured as above with the antenna off.

Electric field measurements were made with a narrow-band, highly sensitive Hewlett-Packard 3581a Signal Analzyer, which measured the voltage induced across copper grounding pegs inserted into the soil with a 1 m separation between them. For source impedance measurements, 8060A Portable voltmeter was used with the same set of grounding pegs, and varius known resistances were placed across the meter. Magnetic field measurements were made with the Hewlett-Packard instrument and a ferrite-core induction coil. All systems had been calibrated by the IITRI group. The results of the measurements are summarized in the accompanying table. When outdoor work resumes in the spring of 1983, the fields will be remeasured and a single control and ground site will be chosen. The criteria will include stability of the field measurements, stability of the natural setting, and accessibility, the latter being because one ground site is served by roads that may flood.

Table I

Site	Parameter	N-S On	E-W On	Ant. Off
Antenna	E-parallel	0.150 V/m	0.006	9×10^{-5}
	E-perpendicular	0.010 V/m	0.002	2×10^{-5}
	E-source			
	H-parallel	0.011 G	0.001	1×10^{-6}
	H-perpendicular	0.044 G	3×10^{-4}	8×10^{-6}
	H-vertical	0.124 G	0.004	3×10^{-5}
Ground #1	E-parallel	0.090 V/m	0.002	9×10^{-5}
	E-perpendicular	0.93 V/m	0.03	9×10^{-4}
	E-source Z			
	H-parallel	7×10^{-4} G	2×10^{-5}	6×10^{-7}
	H-perpendicular	2×10^{-4} G	6×10^{-5}	2×10^{-6}
	H-vertical	0.002 G	6×10^{-5}	5×10^{-6}
Ground #2	E-parallel	0.005 V/m	0.137	4×10^{-5}
	E-perpendicular	0.102 V/m	3.6	2×10^{-4}
	E-source			
	H-parallel	1.4×10^{-5} G	4×10^{-4}	1×10^{-6}
	H-perpendicular	8.6×10^{-5}	0.003	5×10^{-7}
	H-vertical	1.0×10^{-4} G	0.0037	5×10^{-7}
Control #1	NS	8×10^{-4} V/m	8×10^{-4}	5×10^{-5}
	EW	8×10^{-5} V/m	2×10^{-6}	3.6×10^{-5}
	E-source Z (not measured)			
	NS	4×10^{-6} G	3×10^{-6}	1×10^{-6}
	EW	7×10^{-6} G	9×10^{-6}	1×10^{-6}
	H-vertical	4×10^{-6} G	5×10^{-6}	1×10^{-6}

Table 1

Site	Parameter	N-S On	E-W On	Ant. Off
Control #2	NS	2.2×10^{-4} V/m	2.4×10^{-4}	0.0015
	EW	7×10^{-4} V/m	5×10^{-4}	9×10^{-4}
	E-source Z (not measured)			
	NS	6×10^{-6} G	6×10^{-6}	1.6×10^{-5}
	EW	3×10^{-6} G	5×10^{-6}	4×10^{-6}
	H-vertical	2×10^{-6} G	2×10^{-6}	6.4×10^{-5}

Note: Ground #2 located on E-W arm, antenna and Ground #1 located on N-S arm.

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2. Goodman, E. M., B. Greenebaum, and M. T. Marron, 1979. Effects of extremely low frequency electromagnetic fields on Physarum polycephalum. Variation with intensity, waveform, and individual or combined electric and magnetic fields. Radiat. Res. 78: 455-501.

3. Goodman, E. M., and T. Beck, 1974. Metabolism during differentiation in the slime mold Physarum polycephalum. Can. J. Microbiol. 20: 107-111.

4. Bradford, M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-56.

Glossary - Acronyms

Respiration:
A measurement of the rate of oxygen utilization.

Antenna ground:
A conducting connection between the transmitting antenna and the earth.

Axenic culture:
Growth of a single organism (slime mold) in the absence of contaminating organisms such as bacteria, fungi, etc.

Oatmeal-agar:
A growth medium containing oatmeal, water in a gelatin-like state.

Plasmodium:
A mass of protoplasm visible to the eye containing numerous nuclei; the entire structure is delimited by a plasma membrane. In the laboratory it is usually maintained on a solid substrate such as agar or filter paper.

Micro-plasmodia:
Plasmodia maintained in submerged shake flasks.

Shake flask cultures:
A method of maintaining plasmodia in a liquid nutrient medium. The flask is continuously shaken to provide oxygen to the culture.

2. Frontispage

(a) Subcontractor's name and address:

  Rudolph Neal Band
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  Michigan State University
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(b) Subcontract number: E06516-82-C-20015

(c) Title: ELF Communications System Ecological Monitoring
             Program, Task 5.2, Soil Amoeba.

(d) Reporting year: July 8 to November 8, 1982 (revised 18APR83)
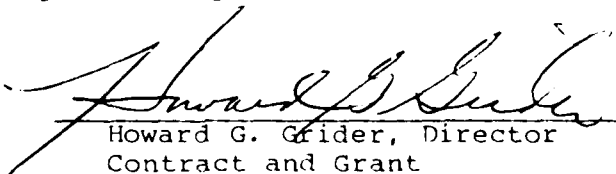
(e) --

(f) Name and signature of principal investigator:

_____ 4/15/83
           Rudolph Neal Band, Principal Investigator

(g) Co-investigators: None

(h) Name and signature of subcontractor's approving and
     releasing authority.

_____
           Howard G. Grider, Director
           Contract and Grant

3. Table of Contents

4. Abstract

Consistent with the contract requirements and the Work Plan, a research facility was established near the proposed research area. Control soil plots were characterized and samples of soil were taken to isolate and characterize amoebae this winter. Equipment was ordered and data management programs were written.

5. Summary

Amoebae are common soil organisms present in large numbers (i.e. ca. 4,000 per gram soil). Ecologically their role in soil formation from organic matter (i.e. mineralization) is to eat bacteria and fungi. In turn they are eaten by others. A gram of soil typically contains at least three species of amoebae (that add up to 4,000 individuals) so that they are doing different things together in a small soil unit. Additionally a lot is known about the physiology of soil amoebae which makes them easy to work with.

Any ecological stress, such as might be induced by the ELF antenna and ground wire, would be reflected in detectable changes in the soil amoeba population. Changes would include numbers of active amoebae, species diversity, their physiology and their distribution in soil. Consequently, the activity of amoebae in soil away from the antenna will be compared with amoebae from similar soil near the antenna. The preliminary part of the work includes setting-up, characterizing soil plots and isolating soil amoebae.

INTRODUCTION

A. Objectives:

Soil amoebae play a significant role in soil mineralization, acting as micro-predators and serving as food for other soil organisms. Since several soil amoebae are used in cell and molecular biology, much of the biochemical and physiological mechanisms underlying their biology is known. Thus, possible electromagnetic effects on animal cells would be readily detected in these organisms.

Possible stress induced by electromagnetic fields might act directly on the organism. Goodman et al., (1976) published evidence of an effect on the cell cycle and cytoplasmic streaming of the slime mold Physarum, an amoeboid organism that lives in rotten wood. Friend et al. (1975) found that the freshwater amoeba Chaos chaos oriented to an electromagnetic field. Thus, the electromagnetic field may exert effects on basic cell functions. An electromagenetic field could also exhibit an indirect effect by acting on other soil components important to the amoebae. Consistent with the recommendations of the National Research Council (1977), to follow up the studies on amoebae and to focus on a general ecological study by the electromagnetic field since these would be at the ecological level. If specific effects are observed this might warrant later examination at the molecular level. The proposed studies because of the earlier Physarum study (Goodman et al., 1976).

Environmental changes in soil that take place over time (e.g. moisture, temperature, etc.) are important to this study. Electromagnetic effects might be restricted to a biological state

induced by a particular environment. Obviously environmental changes will also have a direct effect on soil amoebae.

B. Background:

1. Taxonomy and biology of amoebae found in soil:

The following is a brief description of the common species of soil amoebae: (a) Acanthamoeba castellanii: a free-living species isolated from freshwater and soil. Although strains have similar features, including the same %GC in their nuclear DNA (Band and Mohrlok, 1973), there are distinct features, for example: strain HR agglutinates with concanavalin A; strain MP clumps in fluid culture and contains a unique glycolipid in its surface membrane (Hoover, 1974); strain AN has a sucrase; strain AC is capable of growth at a lower glucose concentration than the other strains. Adam (1964) has found differences between some strains in terms of amino acid requirements and drug sensitivity. All of these differ in terms of their net surface charge density (Band and Irvine, 1965). Immunological differences between some strains are minimal but significant between species (Visvesvara and Balamuth, 1975). (b) Acanthamoeba culbertsoni: capable of growth at elevated temperature; isolated from mammalian tissues and from soil as free-living amoebae. Several strains have been isolated. The %GC of at least one strain differs from A. castellanii (i.e., strain A-1).

(c) other acanthamoebae: Page (1967) revised the taxonomy of the genus Hartmanella, which resulted in some species being changed to the genus Acanthamoeba.

Acanthamoeba polyphaga was described by Sawyer (1970) as a

free-living amoeba. From species descriptions, A. polyphaga is so similar to A. castellanii that the two species could be easily confused although it does differ immunologically from A. castellanii (Visvesvara and Balamuth, 1975). Another species described based on immunological differences is A. royreba (Willaert et al., 1978).

Other species include the larger acanthamoebae A. astronyxis and A. commandoni (e.g. Pussard, 1972), but they differ morphologically from A. castellanii.

(d) Naegleria gruberi: free-living amoebo-flagellate with non-reproductive flagellate stage. Common in soil and freshwater. Several strains have been described.

(e) Naegleria fowleri: this is the name given by Carter (1970) for pathogenic Naegleria. Strain designations are used for isolates. Visvesvara (personal communication) has established a hybridoma for N. fowleri, which will be very important in the clinical identification of this species.

(f) There are numerous other small amoebae: Vahlkampfia (identical to Naegleria but without a flagellate state), Tetramitus (another amoeboflagellate) commonly isolated from animal excreta. Since any colorless, amoeboid organism (without a flagellum) is included among the naked amoebae, this is obviously a polyphiletic assemblage.

The cellular slime mold amoebae (acrasieae) will not be sought out in this study although their dormant cysts occur in soil. These are a group of organisms found in forest litter in smaller numbers than amoebae in soil (e.g., less than 1,000/g,

Cavender & Raper, 1965b). The enrichment procedure for small soil amoebae (modified from Singh, 1946) contains a greater nutrient concentration than that used to isolate cellular slime molds (Cavendar & Raper, 1965a); I understand from discussions with investigators who work with cellular slime molds that the higher nutrient concentration would inhibit growth of their organisms. I have not encountered them in soil enrichments but attention will be paid to their possible presence.

Present methods of distinguishing species and strains include immunological differences (e.g. Visvesvara & Balamuth, 1975); differences in mitochondrial DNA (Byers, personal communication); isozyme differences (e.g. Daggett & Brooks, 1975); net surface charge density differences (Band & Irvine, 1965). To date all of these genetic markers have not been fully exploited to supplement morphological characters in an over-all taxonomy of small amoebae. However, these techniques are being used within subgroups and they are equally applicable to soil amoebae as a whole.

2. Morphology and life cycles: The general form of the amoeba state differs between genera: Naegleria exhibits a smooth outline (except the collopod region) and moves rapidly while Acanthamoeba exhibits numerous fine pseudopodia and is lethargic in movement. Sex has not been reported in either genus. Reproduction by binary fission is superficially similar, both requiring a surface for cell division. Nuclear mitosis in Acanthamoeba is similar to animal cells except for the absence of the centrioles. In those species examined, the centriole is either replaced by a small, "dense" body or a large centrosphere. In Naegleria the nucleolus divides at

mitosis, the nuclear membrane is retained and discrete division centers (i.e. centrioles) are not observed. _Naegleria_ does not round up at mitosis, as opposed to most animal cells, but continues to locomote during nuclear mitosis. _Naegleria_ will transform into a non-reproductive, flagellate stage (e.g. Fulton, 1970; Schuster, 1979).

Both _Naegleria_ and _Acanthamoeba_ have a dormant, cyst stage. _Acanthamoeba_ has a wrinkled, bilayered cyst wall without obvious pores (evidence for a plugged pore can be seen in the electron microscope, Bowers and Korn, 1969). _Naegleria_ has a smooth round cyst with pores visible in the light microscope. The number of pores differs within the genus (_N_. _gruberi_ has more than _N_. _fowleri_).

3. _Nutrition and physiology_: amoebae are phagotrophs, engulfing solid food and digesting it in food vacuoles. Food preference studies on _A_. _castellanii_ (e.g. Singh, 1941; Heal, 1963) indicate that, in a mixture of bacteria, only certain bacteria are eaten and that some yeasts are also eaten while fungal hyphae are not. In common with many micro-organisms, the amoebae eat blue-green algae as well (Wright, _et al_., 1981).

The nutritional requirements for several strains of _A_. _castellanii_ have been established. Thiamine, B_{12} and biotin are required for growth as well as a carbon source (e.g. glucose), amino acids and inorganic nutrients. The amino acid requirement differs between strains, but in general consists of less than a dozen amino acids (Adam, 1964; Band, 1962; Byers _et al_., 1980). The concentration of nutrients required for growth by acanthamoebae is

roughly ten-fold higher than most cells (protozoan and metazoan).
All of the acanthamoebae are obligate aerobes that can grow
normally under CO_2-free air or under elevated CO_2 tensions
(i.e., 10% for those strains that I have tested). This may have
important ecological consequences in view of soil's elevated CO_2
content. The amoebae grow best at pH 6 to 7 although cultures
started at pH's between 5 and 6 grow satisfactorily and enough
ammonium is excreted during growth to change the pH to the 6 to 7
range rather quickly. The amoebae can tolerate acid pH's better
than alkaline, although both are deleterious over time. Osmotic
pressures greater than 0.2 osmolal inhibit growth.

Although Naegleria can be cultured in axenic media, devoid of
living or dead bacteria, a defined medium has not been established
as yet. Present axenic culture media contain liver extract, yeast
extract and a peptone or some combination of these with heme (Band
and Balamuth, 1974). The culture medium for Naegleria is too
dilute to support growth of Acanthamoeba. Further, Naegleria is
able to grow at much greater osmotic pressures than Acanthamoeba.

The effect of temperature on survival of several strains has
been published (Griffin, 1972). Most of the small amoebae fall into
two groups, those that survive in the 30 to 35°C range and those
capable of survival at somewhat higher temperatures. The latter
group includes non-pathogenetic and pathogenic strains. Admittedly
most soils do not reach temperatures such as these.

4. Ecological considerations: distribution studies of small,
free-living amoebae in soil have been carried out over many years
(e.g. Cutler et al., 1922; Sandon, 1927). Stout and Heal (1967) and

Heal (1971) have published reviews on soil amoebae. The published number of amoebae in soils range from 2 to 22 X 10^3/g soil for unfertilized clay loam (Singh, 1949) to 2 to 51 X 10^3/g soil for cultivated sandy loam and 12 to 180 X 10^3/g soil for the rhizosphere in sandy loam (Darbyshire and Greaves, 1970). Singh (1949) found a range of 14 to 146 X 10^3/g soil in manure fertilized clay loam.

The small amoebae are thought of as micropredators, feeding on bacteria (Singh, 1964). Clarholm (1981) studied changes in bacterial and protozoan population in soil after a rain and concluded that amoebae were the primary regulators of bacterial populations in soil. Acanthamoeba feeding studies indicate selective feeding on bacteria. Singh (1941) found that the exo-toxin in Serratia marcescens prevented phagocytosis of bacteria by the amoebae. Singh (1942) observed that Rhizobium is not eaten by amoebae. On the other hand, Danso and Alexander (1975) reported several genera of amoebae capable of feeding on Rhizobium. Singh (1945) found that chromogenic bacteria were toxic to the amoebae. Acanthamoeba can also use yeast as a food source (Heal, 1963).

Darbyshire and Greaves (1967) found an increase in the number of amoebae in the rhizosphere as opposed to surrounding soil but did not observe a qualitative difference in the type of amoebae. On the other hand, Geltzer (1963) observed a greater diversity of amoebae in the rhizosphere.

Tolerance of amoebae to most physical factors in soil are known from in vitro studies. Naegleria and Acanthamoeba are obligate aerobes although the dormant cyst can survive under

anoxia. I have not observed growth inhibition with 10% CO_2. The amoebae are not pigmented and studies have failed to demonstrate light effects (e.g. growth inhibition, entrainment, etc.). However, the freshwater amoeba Amoeba proteus exhibits negative phototaxis (Mast, 1932) although it is "colorless", so light effects cannot be ruled out for the small amoebae. An interesting temperature range for the small amoebae lies around 20 to 25°C. In the laboratory some strains do not grow at a significant rate in this temperature range while others do. For example, A. culbertsoni, strain A-1 grows fairly well at 37°C, quite well at 32°C and hardly at all at 25°C. From temperature data used in texts and reviews (e.g. MacFadyen, 1967), even surface temperatures of bare soils in mid-summer hardly make it up to 25°C, let alone at deeper levels. There are obvious differences to this, e.g. in hotter portions of temperate climates at mid-summer, where 32 to 37°C would not be an exception, but for most temperature climates maxima of this sort do not hold for any depth and are mediated by a variety of factors (e.g. ground cover, air movement, soil moisture, etc.). The implications of this are interesting in terms of growth at different seasons of the year and in terms of stratification within a given area.

Obviously moisture becomes a limiting factor for amoebae in soil. A protozoan organism in soil survives by remaining in water-filled pores (Darbyshire, 1975). Soil texture and moisture interact in terms of the distribution of pore sizes. Losina-Losinsky and Martinov (1930) found that the movement of Vahlkampfia in garden soil related to moisture content. At 25%

water, the amoebae were limited to the top 0.1 mm of soil. However, this was finely dispersed, sterile garden soil. Although I have not examined vertical movement, I can find amoebae in sandy loam with a similar water content at much greater depths. Using millipore filters, I find that the amoebae can penetrate water-filled pores of 1.2 μm diameter. Further, amoebae remain vegetative but cannot grow at 1 bar suction in soil. Using Darbyshire's (1975) assumptions in his study on Colpoda in soil, this would mean that the amoeba requires pores 3 μm in diameter to be water-filled. This pore is less than than the diameter of a round amoeba but larger than the 1.2 μm an amoeba can crawl through. This consistent with Darbyshire's (1975) proposal that water-filled pores are required by protozoa in soil.

Although the position of small amoebae in a food chain has not been established, several biological interactions have been reported. Nikoljuk (1969) reported that the amoeba's metabolic products include a plant growth substance (i.e., indolyl-3-acetic acid) as do other soil microbes (Mosse, 1975). Darbyshire and Greaves (1970) found an increase in bacteria in the rhizosphere when acanthamoebae were present. Miles (1963) found that protozoa were required in the earthworm diet. Habte and Alexander (1975) reported evidence to indicate that protozoa are capable of cropping the plant pathogen Xanthomonas compestris. Danso and Alexander (1975) noted that amoebae can crop bacterial populations in soil effectively but cannot eliminate the bacteria. Gould et al. (1979) found that plant root acid phosphatase production was affected by bacteria and amoebae. Verma, et al. (1974) found that Acanthamoeba
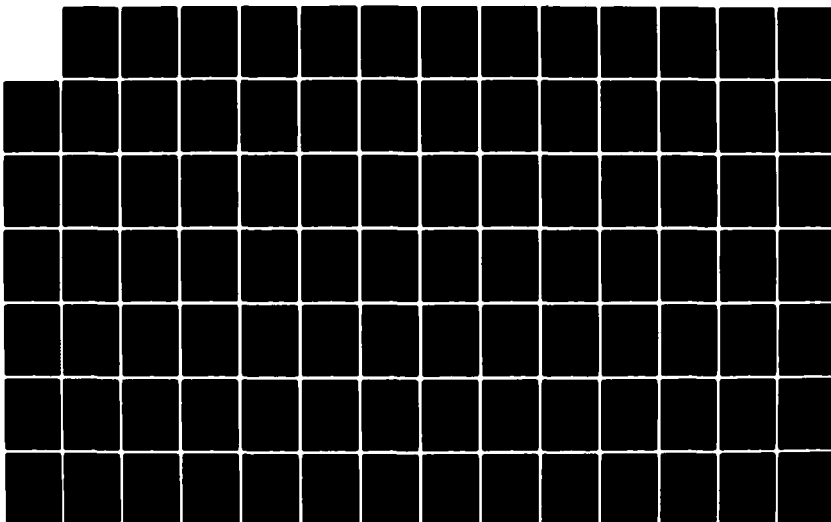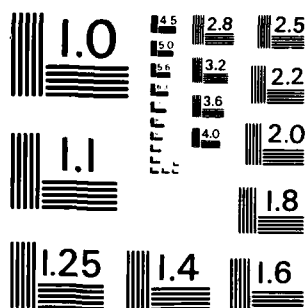
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NATIONAL BUREAU OF STANDARDS – 1963 – A

cysts are eaten by a soil fungus in conjunction with a soil bacterium.

5. Unpublished related research: Core samples (litter and soil, 7 cm and 15 cm deep) were taken from 12 sites over a one year period at the Rose Lake State Wildlife Research Area near East Lansing. The sites were chosen to include a variety of mineral and organic soil types. All sites contained Acanthamoeba, Vahlkampfia and a small, unidentified amoebe. Naegleria was present consistently at only 4 sites (3 mineral soils and 1 organic soil). There was no obvious correlation betwen Naegleria distribution and soil type or litter (e.g. grassland, deciduous wood lot, etc.) nor was there a correlation to soil properties (e.g. porosity, organic content, pH, etc.).

In addition to establishing a heterogeneous distribution of Naegleria in this locale, Singh's differential counting method (Singh, 1946) was used so that data was also obtained on the number of vegetative amoebae vs. dormant cysts in each sample. The data indicated that vegetative amoebae predominated during the season of active plant growth. At other times amoebae existed in the cyst stage. Further, vegetative amoebae were present in greater numbers in the underlying soil (i.e. 7cm and 15cm depth) than in the litter. Where Naegleria was present, together with other amoebae, its numbers ranged up to 16,000/g soil.

The isolation of several amoebae from a small quantity of soil is not unique, similar observations were made on cellular slime molds in forest litter (e.g. Cavender & Raper, 1965a, b, c, 1968). Eisenberg (1976) studied the two-dimensional microdistribution of

SOIL

| ANTENNA | CONTROL(S) | GROUND WIRE |

FIELD WORK

| STRATIFIED RANDOM SAMPLES | CLUSTER ANALYSIS | IN SITU EXPERIMENTS: 1. Cropping 2. Cell cycle | AMBIENT MONITORING: 1. On site 2. External |

ON SITE LAB

SOIL ENRICHMENT CULTURE AND COUNTS

| POPULATION SIZE: cyst & vegetative | CLUSTER ANALYSIS COUNTS | DATA STORAGE & INITIAL PROCESSING |

PRELIMINARY SPECIES SCREENING

SPECIES ISOLATION

CELL CYCLE: autoradiography analysis

MSU LAB

SPECIES CHARACTERIZATION: morphology; genr'l physiol.; immunology; mitochondrial DNA; net surface charge density

IN SITU EXPERIMENTS: isolate & characterize species common to exptl. & control sites

to FIELD WORK

DATA ANALYSIS

Figure 1. Flow sheet of proposed research for soil amoeba task (5.2).

cellular slime molds to further demonstrate their coexistence in soil. Horn (1971) found that different species of cellular slime molds isolated from the same soil differed in their food preference, thus providing a basis for their coexistence in soil.

METHODS

A summary of the proposed research is given in Fig. 1. Certain components feed back, these will be noted. Data analysis will be structured so that it will be compatible with data from the other Task Groups where possible. Blind scoring will be used. The intent is to prevent bias on the part of the student workers and technicians, samples will be identified by the P.I. with random numbers.

A.   Environmental monitoring and site selection:

Paired sites will be selected with the assistance of IITRI or the contractor responsible for ambient monitoring of electromagnetic fields. Soil plots will be chosen near the antenna and near the ground wire to comply with the specified field strengths and magnetic effects. Control plots will be chosen to assure a drop of at least one or two orders of magnitude in electromagnetic effects, including other sources of radiation (e.g. 60Hz). Plot sizes will be as large as possible while maintaining environmental uniformity so that the same plots can be used for the duration of the study if possible. Since the exact location of the antenna may not be known during the first growing season (1982) and some empirical testing

will be involved in selecting plots the first year, plots may have to be changed in the subsequent growing season.

Meteorological, ambient monitoring important to this Task are soil moisture and temperature. Automatic sensing will be used to record soil moisture and soil temperature in the litter layer and at two soil depths. Soil chemistry and characterization of fixed soil properties (e.g. soil suction, soil classification, etc.) will be included to assure that control and experimental plots are similar. Soil classification and chemistry will be done by MSU's Soil Science Department. Soil chemistry differs from stable soil properties (e.g. soil profiles, soil classification, soil suction, plant cover) in having the potential to change seasonally. Four times during the growing season it is planned to pool 20 random samples from each plot for chemical analysis. Analyses will include: P, K, Ca, Mg, Mn, Zn, Cu, Fe, Na, Cl, total N, nitrate, organic matter. Soil suction measurements, plant cover characterization and soil horizon descriptions will be performed by the Principal Investigator. Ideally only one control plot is needed to compare antenna and ground wire plots with a three-way anaysis of variance in comparing experimental data. However, it may not be possible to obtain three plots of this sort. Consequently, in planning the proposed research, I assume that two control plots, one for each experimental plot, may be needed to be certain so that there will be similar soils in experimental and control sites. Therefore, paired plots will be used, one control for each experimental plot. Sampling from plots will be done in pairs.

Statistical analyses will be done with either the t-test or analysis of variance.

The first growing season (1982) will be exploratory in nature. Aside from establishing sites, soil samples will be used to look at details of amoeba population size and clustering analysis as a site selection tool rather than as a consistent monitoring tool. The first season will be important in determining the periodicity of soil sampling, the number of samples taken and the sample size.

B. Plot sampling:

Part of the objective of this proposal is to determine the effect of the electromagnetic field on the number of amoebae in soil, the ratio of dormant to vegetative organisms and the number of amoeba species in soil. Changes due to other environmental factors and to unknown factors will be detected with control plots, ambient monitoring and careful sampling of the population over time during the growing season. Clustering of soil and litter organisms is well established (e.g. Horn, 1971). The manner in which the amoebae are clustered in soil as a whole and by species over time will have a major influence on techniques used to determine population size and species number. Additionally, the soil component responsible for clustering of amoebae may be affected by the electromagnetic field, and this would change the pattern of clustering. Therefore, changes in clustering patterns in themselves will be important to monitor in control and experimental plots.

Initially soil plots approximately 10 m sq. will be set-up in 1 m sq. grid patterns for random sampling. This will provide space for the first season. For the long-term study, I would prefer 1 acre

plots. A minimum of two, random samples will be made with a presterilized 1 inch coring tube and the upper, distinct horizons separated for counting, in each plot. Litter will be discarded. Vegetative amoebae are present in the greatest number during the growing season in the organic and mineral topsoil layers. Samples are best counted on the same day they are taken, vegetative amoebae rapidly encyst and both cysts and vegetative amoebae die from predation and other causes. Cluster analysis will help in determining the number of samples needed, beyond a minimum of 2 per plot at a given sampling interval. Sampling interval (i.e. daily, every two days, weekly) will vary during the season, depending on growth activity that ranges from complete dormancy during the winter to active changes during the peak of the growing season. The first growing season's data will determine work effort for subsequent years. As an initial approximation, I assume the sampling interval will be every other day during the peak growing season and a final season's sample when the soil freezes.

C. Cluster analysis:

The spatial distribution of amoebae in soil will be determined by quadrat sampling (Morisita, 1959; Pielou, 1969; Hairston et al., 1971). It is used in ecological studies of dispersion and distributional patterns, and it has been used as well to study the distribution of cell types in a tissue (Smith-Gill, 1975). The size of the sample unit (quadrat) is varied from small quadrats that are smaller than a cluster of organisms, and larger. The technique distinguishes between randon, clumped and uniform distributions of organisms. Although uniform sampling is desirable, the small quadrat

sizes needed for this study will dictate using random samples. To test for an association of two species, quadrat size must be small so that the quadrats do not contain both species in every sample. Since I can isolate three species from 0.1 g of soil, the quadrats will be very small.

D. Soil enrichment culture and counts:

Simultaneous detection, preliminary identification a.d differential enumeration of vegetative amoebae vs. cysts will be done by the method of Darbyshire et al., 1974 with 15 two-fold dilutions and 8 replicates per dilution. I have modified this by using dilute nutrient medium (DSG) and by killing vegetative amoebae, for cyst counts, with 1% sodium dodecyl sulfate (SDS) rather than 2% HCl originally used by Singh (1946). The DSG medium contains: 2.13g, $MgCl_2$ $6H_2O$; 0.136g, KH_2PO_4; 0.568g, Na_2HPO; 1g Trypticase (BBL), 1g yeast extract (BBL), 1g glucose, and distilled water to 1L. The saline (LS) used to suspend and dilute soil, to washout SDS and to suspend the bacterial food organisms is one that I have used for laboratory studies of Acanthamoeba (Band and Mohrlok, 1969): 2.9g NaCl; 0.65g $MgSO_4$; 0.04g $CaCl_2$ and distilled water to 1L. The choice of food organism (i.e. E. coli) is common, although some use Enterobacter. I did compare E. coli with the soil bacterium Arthrobacter crystallopoietes (rod and spherical cells) and with a soil enrichment. All of these enrich the same amoebae from a soil sample but E. coli supports better growth. For the soil enrichment of food organisms, I used a soil extract-DSG medium, relying on the growth of soil bacteria in the sample to support amoeba growth. Since

biological populations are genetically heterogeneous any enrichment procedure is bound to be somewhat selective against a portion of the species population. However, all of the major species of soil amoebae can grow on E. coli. Horn (1971) enriched different species of cellular slime molds from a sample of soil litter with E. coli, although each species exhibited different preferences for bacteria isolated from litter. Darbyshire et al., (1974) relied on soil bacteria to grow amoebae.

To isolate species of soil amoebae, amoebae from multiwells are subcultured onto DSG-agar plates with E. coli, allowed to grow and encyst, subcultured with a loop and speciated. The initial choice of amoebae to subculture is based on cyst morphology. Species of Acanthamoeba can be recognized by cyst morphology. Naegleria and Vahlkampfia both have round cysts that cannot be distinguished. Clonal isolates of these must be examined on the basis of vegetative and cyst morphology and on their transformability to flagellates. Morphological characters of vegetative amoebae, cysts and transformability should be adequate for preliminary speciation. Speciation methods will be given below. For the cluster study, a series of isolates from different soil sample sizes will be scored for the number of amoebae of a given species. It will be important to prove that morphologically identical species from different sample sizes are the same species.

E. Species isolation and characterization:

As stated earlier, preliminary speciation can be done by morphology of amoebae and their cysts. Clone cultures will be established on plates, using E. coli as the food organism; where

possible these will be put into axenic culture. Vegetative amoebae and their cysts will be characterized on the basis of their morphology, growth properties, net surface charge density, restriction fragment patterns of isolated mitochondrial DNA, and isozyme patterns. After the preliminary phase of the work has been completed, it may be possible to use fewer of the above properties to characterize the species. However, it is very important that species and strains of species by precisely defined for this study. For example, if control and experimental plot isolates of similar amoebae actually contain different strains, these might behave differently between plots irrespective of an electromagnetic field. Subtle changes in species/strain diversity may occur over time with or without the electromagnetic field. When a clone isolate is selected as common to control and experimental plots, for reintroduction into the plots for cropping and cell cycle analysis, it is essential that differences not be strain differences. Aside from the use of replicate cultures, apparently identical strains from control and experimental plots will both be tested, side-by-side, in control and experimental soils.

The morphology of the amoebae will be determined with the light microscope, representative amoebae will be preserved for the record. Net surface charge density (Band & Irvine, 1965) requires little instrumentation and it is a quick procedure that will be done at the site lab as part of the preliminary species screening. Mitochondrial DNA from several strains and species of small amoebae have been studied recently by Dr. T.J. Byers at Ohio State University (personal communication). Restriction endonucleases were used to

obtain fragments for electrophoresis (Lansman et al., 1981) and the electrophoresis patterns were analyzed by the method of Engels (1981). Isozyme patterns have been used to examine strain and species differences among small amoebe (e.g. Daggett & Brooks, 1980).

F. Cropping efficiency:

Cropping efficiency is measured by determining the growth of amoeba isolates in a non-nutrient saline containing different number of food bacteria. Counts are made of changes in amoeba number and bacterial number over time. An example of this method is seen in the paper by Danso & Alexander (1975). Bacterial counts can be done by the most probably number method and the amoebae will be directly counted with a hemacytometer. The food bacterium will be a bacterial isolate from the soil plot that supports growth of the test amoeba. Test amoebae will be isolated from the soil plots. The same species of amoeba from control and experimental plots will be studied. Replicated laboratory incubations and field incubations will be tested. For field incubations, cultures will be incubated buried in control and experimental plots. Clone isolates from both control and experimental plots, that appear to be the same strain, will be incubated side-by-side in both control and experimental plots.

G. Cell cycle:

The cell cycle of an Acanthamoeba isolate will be determined by the methods previously used with this organism (Band & Mohrlok, 1973) if differences in other growth parameters are noted between control and experimental soils. This is the classical procedure using tritiated thymidine incorporation followed by an

autoradiographic analysis of metaphase chromosomes over time. Other parameters of the cell cycle are obtained by cell counts and phase contrast microscopy (i.e. growth rate, mitosis time). I plan to do the experiments with amoebae incubated in axenic culture medium buried in test and control soil plots. Long term effects will be observed by studying amoebae isolated from and incubated in test and control soil plots. Amoebae in buried cultures can be pulse labelled in the field, chased with cold medium with a hand centrifuge in the field and fixed on glass slides. Subsequent washes and autoradiography (in a darkroom) can be done later. The cell cycle will be determined independently for apparently identical clones isolated from control and experimental plots and incubated side-by-side in control and experimental plots.

H. Performance sites:

Figure 1 gives the performance sites. I will attempt to concentrate my efforts on the field work during the growing season and delay work at MSU until the dormant season. It will be necessary to do the soil counts immediately after sampling to obtain ratios between vegetative amoebae and dormant cysts, and to assure survival of encysted amoebae. Encysted amoebae survive for long periods in vitro, but in soil cysts are eaten by other microbes as discussed earlier in this proposal.

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WORK PLAN

The following is an exerpt from the original work plan submitted to IITRI with the first monthly progress report (July, 1982):

Period: July 1, 1982 to October 31, 1983

Element 1 - Research facility

> Synopsis - Rent an on-site research facility, obtain equipment and supplies needed to perform research.

Element 2 - Plot selection and characterization

> Synopsis - Survey area to identify potential study soils, near proposed locations for the antenna and the ground wire, and a control area away from both.

Element 3 - Ambient monitoring system

> Synopsis - Calibrate temperature and moisture probes for the study area soils, install and use during 1983 growing season.

Element 4 - Species and strain characterization

> Synopsis - Using morphological and physiological markers, identify species and strains of soil amoebae from the study area.

Element 5 - Biological diversity and activity

> Synopsis - During growing season, determine ratio of

vegetative to dormant amoebae, to species
and strain, as it relates to ambient
monitoring and to spatial distribution.
Growth rates and cropping activity will be
determined.

Element 6 - Data management

Synopsis - When enough data is obtained, it will be
analyzed statisticaly for possible
correlations to environmental variables and
to other biological data (from other
tasks).

SCHEDULE:

Month

Element	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	X	-	-	-	-	-	X									
2	X	-	-	-	-	-	X									
3					X	-	-	-	-	-	-	-	-	-	-	X
4					X	-	-	-	-	-	-	-	-	-	-	X
5											X	-	-	-	-	X
6					X	-	-	-	-	-	-	-	-	-	-	X
Reports	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

6. PROGRESS

Work plan elements 1 and 2 consist of obtaining an on site research facility, obtaining equipment and supplies and selecting soil plots. To the extent possible without knowledge of the antenna site, these work elements were done. Two research sites were selected, 12 miles apart that had the same soil type, both in mixed hardwood forests with a dominance of maple trees. The soil type is very common to northern Dickinson county so it is probable that the antenna will pass over this soil type. Both sites can be reached with ordinary vehicles for most of the season, the sites are acceptable to the DNR foresters and they are not adjacent to high tension electric lines. In addition to the forest plots, a plot was established in a meadow adjacent to one of the forest plots. Soil analysis of the 3 plots indicated that they were chemically identical. Samples were taken to characterize amoebae from the 3 sites. It will be useful to determine the diversity between 2 widely separated but similar sites as well as adjacent sites with different plant cover. The selection of experimental sites and the general design will be influenced by the diversity observed between the present sites.

During the winter season other elements of the work plan will be initiated as indicated above. I am currently isolating amoebae from soil samples obtained from the 3 plots. I am also initiating techniques required to characterize the amoebae (e.g. mitochondrial DNA restriction fragments, etc.). A soil

submersible culture vessel has been designed to test the effect of antenna radiation on the cell cycle and feeding efficiency of amoebae *in situ*.

7. <u>Peer Reviewers</u>

   Peer reviewers were contacted but not used for this
report. I will use them for next year's report, they are:

1. Prof. Thomas J. Byers, Ohio State University, Department of
  Microbiology

2. Prof. Fredrick L. Schuster, Brooklyn College, Department of
  Biology
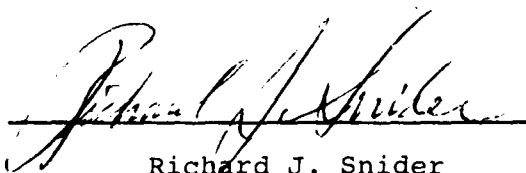
AD P001279

Michigan State University

East Lansing, Michigan 48824
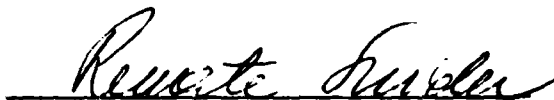
Subcontract No.

E06516-82-C-20015

ELF Communications System Ecological Monitoring Program:

Soil and Litter Arthropoda and Earthworm Studies

Task 5.3. and 5.4.

1982

Richard J. Snider
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Renate M. Snider
Co-Investigator

Michigan State University

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ABSTRACT

Seven potential study sites were located in the ELF system area, all in maple-basswood forest in similar stages of maturity. Field laboratory facilities were established, and equipment for faunal extraction was constructed on site. Arthropod and lumbricid faunas were sampled quantitatively and qualitatively in one of the sites, and approximately 1500 sample units were obtained from August through October. Analysis and identification of this faunal material will yield taxonomic composition, population density estimates, and activity patterns of the soil-litter invertebrate community in maple-dominated forest in the future ELF system area.

SUMMARY

A preliminary survey of selected invertebrate communities
(arthropods and earthworms) of soil and litter was performed
in mature maple-basswood forest near the future location of
the ELF antenna in Michigan's Upper Peninsula. Using several
techniques, earthworms and their cocoons, and litter- and soil-
dwelling arthropods, were sampled from August through October
and preserved for identification. Night-day activity of species
mobile on the forest floor was assessed simultaneously. Ana-
lysis of this faunal material will yield pilot data on compo-
sition, density and activity of a soil-litter invertebrate
community typical of Northern Michigan deciduous forest
ecosystems.

OVERALL APPROACH: TASKS 5.3 and 5.4.

INTRODUCTION

The Extremely Low Frequency (ELF) antenna system in Michigan's
Upper Peninsula is scheduled for construction several years from
now. The exact magnitude and extent of the electromagnetic fields
it will create are not known; it is known, however, that their
effects are potentially more severe for organisms living in contact
with soil (Greenberg 1976).

A similar system has been in operation in Wisconsin for more
than a decade. Questions about its effects have prompted a number
of laboratory studies with test organisms ranging from slime molds
to monkeys (Goodman et al 1976; Medici and Magdaleno 1976).
Isopods and other invertebrates were tested as to their metabolic
rates, which were found unchanged by exposure to ELF (Greenberg and
Ash 1976).

Some field studies at the Wisconsin test facility have been
concerned with monitoring soil arthropod populations for several
consecutive years (Greenberg and Asher 1975; Greenberg 1976).
Small field plots were established in a variety of habitats, from
forest to clover fields, and faunal samples were identified to
order or suborder level (Collembola, Cryptostigmata, Mesostigmata,
Prostigmata). Based on relative abundance of these taxa, Greenberg
(1976) concluded that exposure to ELF radiation for 6 years had not
affected the soil arthropods. Differences between plots, seasons
or years probably reflected natural population fluctuations due to

environmental factors. We suspect that some of these fluctuations resulted from successional changes, which took place "normally" in the plots.

We do not question the validity of these conclusions, restricted as they are to relative frequencies of taxonomically broad microarthropod groups. We do, however, argue that the conceptual approach to such a monitoring program, particularly its level of specificity, must be reversed:

a) Very broad where it concerns the choice of "system": study sites must be truly representative of the ecosystem they are a part of, so that selected processes subject to analysis can be extrapolated and interpretated at the ecosystem level.

b) As specific as possible with regard to the level of taxonomic faunal analysis: exclusion of selected members of the invertebrate community cannot be avoided, but within the limits of chosen goals (e.g. the soil-litter arthropod complex) no member group can be disregarded a priori and all taxa should be identified to as low a taxonomic level as feasible.

In effect, the ecosystem should be treated as the "indicator" to change, since it is the ecosystem which integrates and manifests all influences (whether measurable or not) to which it is subjected. Logistics dictate that we be selective in the scope of our investigation, while adhering to this conceptual framework; we have therefore chosen to center our efforts on the one key sub-system through which the bulk of ecosystem nutrients have to pass if they are to be recycled: the decomposition subsystem.

There are essentially two approaches one can take: process-oriented monitoring of organic matter and nutrient cycling; and in-depth characterization of the subsystem's invertebrate communities which act as process-regulators. Both can furnish sensitive indicators of change or disturbance, and each has its own distinct value in ecological research. Combined in companion studies, they provide mutual validation and greatly increased explanatory power; we will therefore quantify the dynamics of soil-litter arthropod and lumbricid complexes, as well as selected parameters of litter breakdown mediated by the decomposer fauna.

In Michigan forests in the ELF system area, we must be able to detect potential future ELF effects against a background of other, complexly interacting variables. In the soil-litter system, these variables produce spatially heterogeneous distribution of faunal populations and their resources; they also produce temporal fluctuations, which are of two kinds: long-term gradual successional changes; and cyclic seasonal fluctuations which may vary from year to year and on which successional trends are superimposed.

All variables which potentially influence the subsystem under study must be quantified if meaningful data interpretation is to be accomplished in the future. Our objectives therefore encompass simultaneous monitoring of:

- macro-climatic variables
- soil moisture and temperature at several depths;

- composition and distribution of vegetation;

- horizontal and vertical distribution of organic matter, litter and selected nutrients, and their relationship to faunal density and distribution;

- identification of faunal succession in decomposing litter together with litter mass loss and elemental changes;

- quantitative and qualitative analysis of arthropod and lumbricid associations of soil and litter.

SITE SELECTION AND STATISTICAL DESIGN

A survey of potential study sites will lead to establishment of two definitive sites (antenna Test and Control), with electromagnetic radiation levels in the control site at least two orders of magnitude below those of the test site. The sites will be located in maple-dominated forest, chosen such that they possess the greatest possible degree of similarity with respect to vegetational compostion and density, soil type, and topographic characteristics.

Each site will be subdivided into a minimum of 10 quadrats, all of which will be sampled on each sampling date. We thereby obtain time-series data points which enable us to quantify site-specific trends in biotic and abiotic parameters. Most importantly, revisiting all quadrats per date increases the power of analysis of variance and covariance by eliminating an error

term from the statistical model.

Statistical tools to be used fall into two main categories:

a) descriptive statistics which quantify characteristics of
the communities in each site: a variety of equitability, diversity
and dominance indices (Southwood 1978; Mueller-Dombois and
Ellenberg 1974) will be calculated to describe site-specific
communities. In addition, principal component analysis will be
used to evaluate the relative effects of biotic and abiotic system
components on dominant species populations. This procedure is a
form of multivariate analysis, and uses matrices of multiple linear
regression and partial correlation coefficients as its
computational basis.

b) Between-site comparisons: preliminary evaluation of site
(community) similarity can be achieved by directly comparing
various ecological indices. We also anticipate comparing
communities (i.e. quantifying beta-diversity) by the Spatz index
and Sorensen's co-efficient of similarity. Species population
parameters will be subjected to standard tests, e.g. Student't
t-test, after normalizing the data where appropriate.

The major statistical tool for test-control comparison will
consist of analysis of variance; where abiotic variables have been
shown to significantly influence faunal or decomposition
parameters, they will be controlled for by analysis of covariance.

In the mixed ANOVA model detailed below, we assume that two
habitat subtypes will need to be sampled, and we have arbitrarily

set quadrats and sampling periods at N = 10; these are tentative numbers and will be adjusted according to final site outlay and sampling schedules.

Key to Symbols:

H - Habitats (hills - depressions, fixed)

    i = N for H, 2

T - Treatment (Test, Control, fixed)

    j = N for T, 2

Q - Quadrats (random)

    k = N for Q, 10

P - Time periods through the year (fixed)

    l = N for P, 10

Basic ANOVA design:

Source	d.f.	F
H_i	1	MS_H/MS_{HQ}
T_J	1	MS_T/MS_Q
$Q_{(j)k}$	10	
$(HT)_{ij}$	1	MS_{HT}/MS_{HQ}
$(HQ)_{i(j)k}$	10	
P_l	9	MS_P/MS_{QP}
$(HP)_{il}$	9	MS_{HP}/MS_{HQP}
$(TP)_{jl}$	9	MS_{HP}/MS_{QP}
$(HTP)_{ijl}$	9	MS_{HTP}/MS_{HQP}
$(QP)_{(j)kl}$	90	
$(HQP)_{i(j)kl}$	90	

NOTE:

a)   For pre-construction data/year, T (Treatment) is taken as
SITES: this allows analysis of variance of between-site
differences for all measured variables. Not only is it important
to identify these differences, if any, but knowledge of their
magnitude forms the basis for post-construction analysis of changes
in those initially dissimilar variables.

b)   For between-year estimates of variability (pre-construction),
T still equals SITES, and the expanded nested model the includes
these additional factors: YEARS nested within Treatments, Periods
nested within Years, and appropriate interactions (TP and HTP
interactions eliminated).

c)   For between-year analysis of variance after the antenna has
become operational, we assume "2 years pre-ELF" versus "1...n years
post-ELF". The data will then be unbalanced for the mixed model
discussed above, and analysis of variance is best handled by a
general Least Squares procedure (MSU Cyber computer facility).

VEGETATION AND ABIOTIC VARIABLES IN DEFINITIVE SITES

Following site selection along the criteria listed earlier,
site-specific variables will be quantified in greater detail,
essentially for two purposes:

a)   to compare non-faunal parameters between sites, so that
differences in vegetational, abiotic and functional parameters,

if any, are identified and quantified;

    b)   to ensure that site-specific faunal components and their behavior can be correlated to other site-specific parameters; i.e., to obtain an explanatory pre-construction data base (on initially existing site differences) which goes beyond species lists or dominance relationships.

    Most of this data base will be cumulated over the first two years in definitive sites, and will also form the basis for computer mapping of habitat characteristics in each site.

Major variables to be monitored are as follows:

    a)   Macroclimate: precipitation, relative humidity, temperature, wind direction and speed, insolation and barometric pressure (data base shared with task 5.11).

    b)   Soil temperature and moisture at several depths, continuously recorded and transferred to microcomputer via EPROM reader.

    c)   Species composition, density, distribution of tree, shrub and herb layers: quantified by point-quarter and transect methods (Mueller-Dombois and Ellenberg 1974); quadrat-specific stand details and seasonal changes in herb composition are added each year.

    d)   Litter inputs: using 1 m2 litter traps, litter will be collected periodically through the season, separated into major components, and quantified as g/m2 dry mass. For the dominant tree species, elemental analysis of litter samples will be performed

(P, Ca, K, Mg, Mn, Fe, Cu, Zn, Al, Mo) by plasma spectoscopy at Michigan State University.

E) Litter standing crops and soil analyses: both of these are obtained in conjunction with 1/16 m2 faunal samples which are heat-extracted (arthropods) or handsorted (lumbricids). To account for the heterogeneity of the forest floor in the system area, samples are taken in two habitat subdivisions: litter-rich depressions and interspersed hummocks. Soil samples are analyzed for pH, organic matter, and macronutrients. Litter samples, after extraction/ handsorting, yield seasonal standing crop estimates (dry mass/m2), and are analyzed at Michigan State for the above 10 elements.

These data furnish the necessary baseline for functional characterization (organic matter input and decomposition) of the sites, and allow correlation of faunal distribution and quality.

SUMMARY OF MONITORING GOALS

Once definitive sites are selected and ambient monitoring equipment has been installed and calibrated, the monitoring program summarized in Figure 1 will be carried out in test and control sites. All its elements together provide a detailed data base on the soil-litter subsystem: its invertebrate communities, breakdown processes, environmental variables, and the interactions between them.
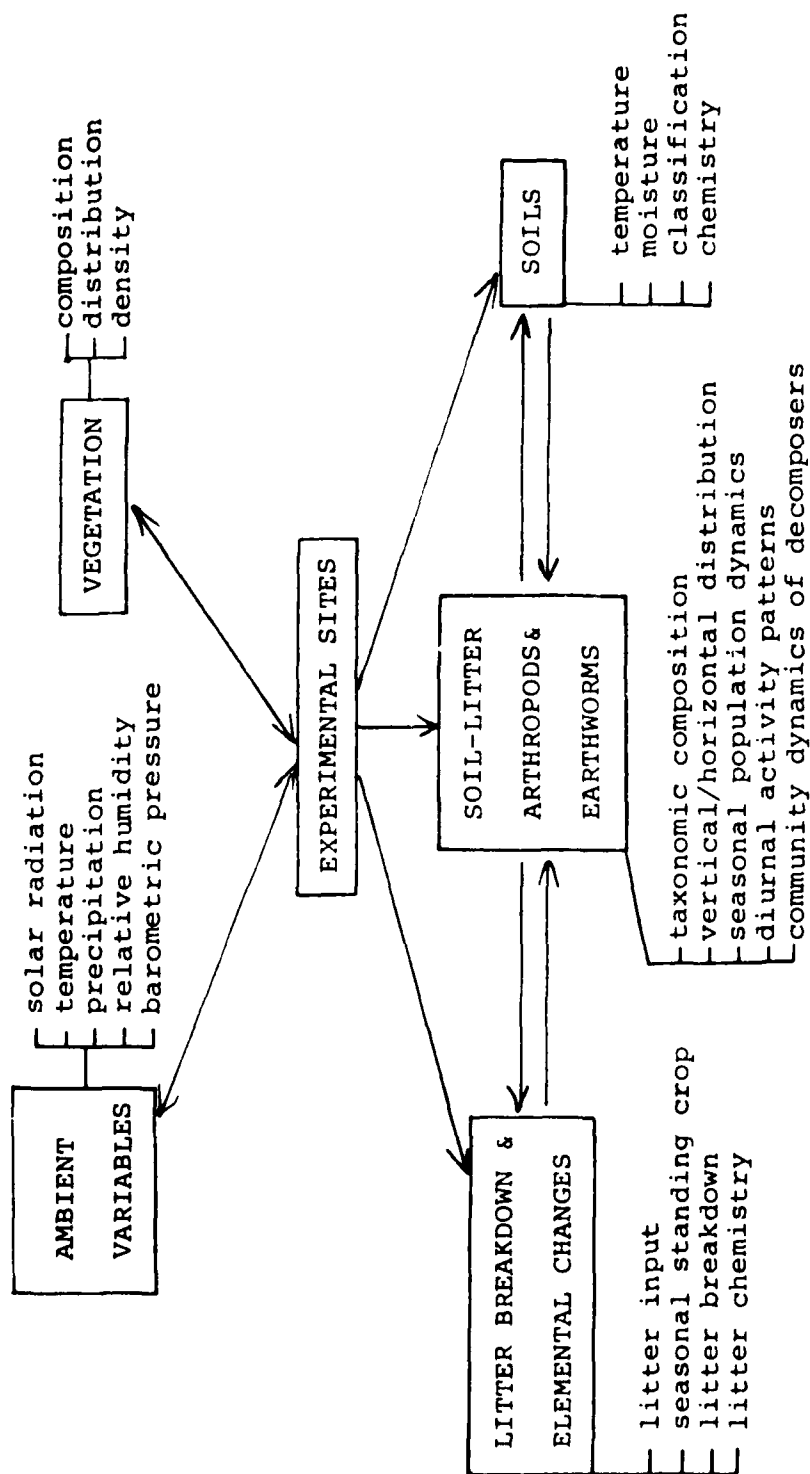
Figure 1. Summary of monitoring goals in Test and Control sites.

ELF REFERENCE COLLETION

It is imperative to assemble a complete collection of specimens from the material accumulated through our various sampling programs. It is useful as a training tool for new personnel, thus facilitating identification of faunal material in the future. In the long term, back-checking on actual specimens may become necessary after the ELF system has been operational for several years.

Finally, permanent preservation of specimens is standard practice in faunal surveys, and is particularly valuable for the Upper Peninsula which is faunistically so poorly known. This colletion, updated every year, will be deposited in the Entomology Museum at Michigan State University.

Below, we list the taxa to be expected at the study sites, and the taxonomic level to which we propose to identify each of them, at least initially. Community analysis may later dictate further taxonomic breakdown of the members of selected orders or families.

TAXON:		LEVEL OF IDENTIFICATION:
MYRIAPODA:	Pauropoda	species
	Symphyla	species
	Chilopoda	species
	Diplopoda	species
CRUSTACEA:	Isopoda	species
ARACHNIDA:	Acarina	family
	Araneae	species
	Opiliones	species
	Pseudoscorpiones	species
INSECTA:	Protura	species
	Diplura	genus
	Collembola	species
	Orthoptera	genus
	Dermaptera	genus
	Psocoptera	genus
	Thysanoptera	genus
	Hemiptera	genus
	Homoptera	genus
	Neuroptera	genus
	Coleoptera	genus
	Mecoptera	genus
	Lepidoptera	genus
	Diptera	genus
	Hymenoptera	genus
	Siphonaptera	genus

DATA MANAGEMENT

Automatically monitored (ambient environmental) and manually collected data (faunal, vegetational, abiotic site characteristics, litter breakdown) are entered into a microcomputer for storage and preliminary statistical analysis. Periodically, they are transferred to a mainframe computer at MSU for final analysis, report preparation and establishment of data archives. The flow of experimental data is presented schematically in figure 2.
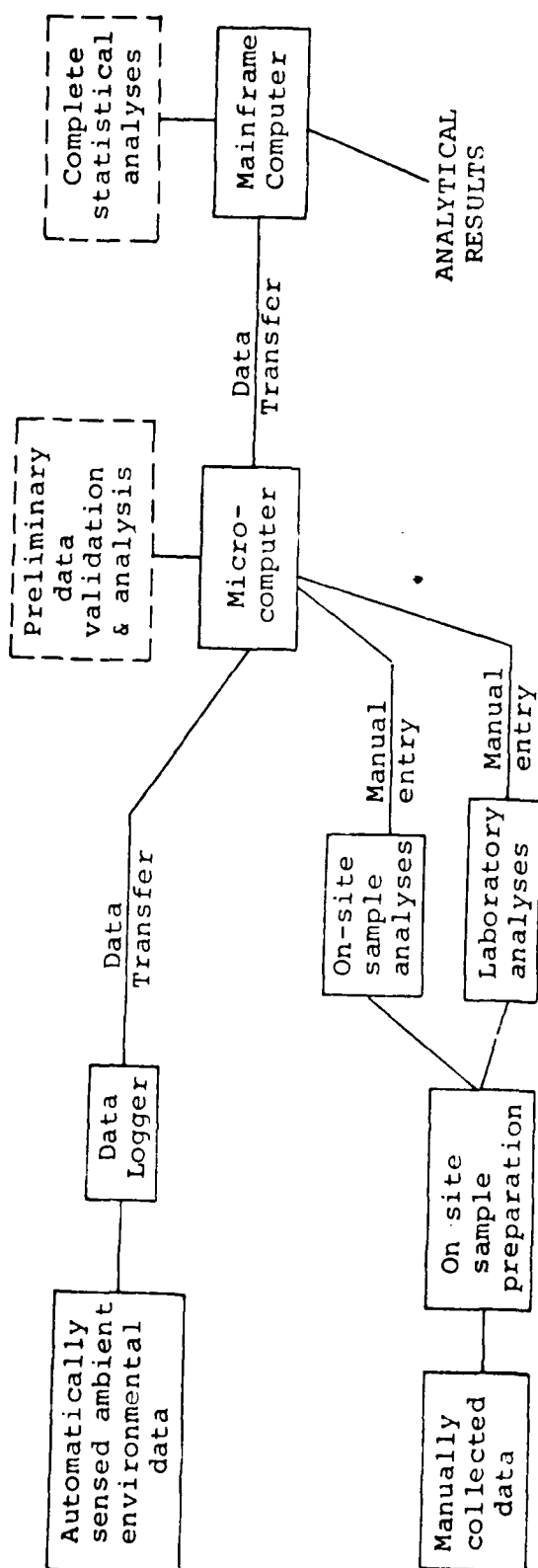
Figure 2. Experimental Data Flow

SPECIFIC OBJECTIVES AND METHODS

TASK 5.3 ARTHROPODA

A.   Species composition, density, relative dominance of arthropods
in soil and litter, and seasonal population age structure of
dominant species:

It has long been recognized that one of the limits to forest
community investigations is the taxonomic level to which organisms
are classified (Wallwork 1970; Swift et al 1979).   In order to
quantify or qualify the role or importance of any given taxon we
must first be able to separate it from its companion taxa.

For many taxa, identification is carried to the species level.
Exceptions are Acarina, which will initially be identified to
family level, and pterygote insects for which preliminary identi-
fication will be carried to generic level.   Based on data analyses,
selected taxa will later be subjected to further taxonomic
breakdown.   Indications that this should be done will come from
three major sources: a) numerical dominance or rarity of a given
taxon; b) suspected or documented functional importance determined
from field observation and/or literature records; c) sensitivity of
a taxon to biotic or abiotic variables (analysis of data from
consecutive years).

Methods: Soil samples (6 cm cores to 15 cm depth) and litter
samples (25 x 25 cm squares) are taken randomly in each quadrat and
heat-extracted in the field laboratory.   Following extraction, a
number of samples will be checked for species-specific extraction

efficiencies by the floatation technique, so that correction factors can be applied to density estimates.

During the winter months, faunal samples are sorted and identified. Species / abundance relationships are then analyzed, and dominant species populations are further broken down into age or developmental classes in order to describe their phenology.

B.   Diel and seasonal activity patterns of surface-active arthropods:

Using pit-traps, we will monitor that segment of the soil-litter fauna which inhabits, or is active in, litter and the soil-litter interface. Strictly speaking, the technique only assesses activity patterns, although Ericson (1979) cautiously suggests that an "approximate" knowledge of the density of dominant species can be obtained by trapping.

We use the technique only for studying activity. For species which are mobile but occur in very low densities (e.g. many large predators) trapping may yield the only reliable description of their phenology. For others, we expect to quantify activity/density relationships by using area-specific sample data (objective A). Such relationships do exist (Ericson 1979), although activity and trapping success are influenced by several other factors which will be monitored at each site. Prominent among them are temperature, rainfall and cloud cover (Joosse 1965; Mobjerg and

Vestergaard 1975; Solem and Sendstad 1978; Ericson 1978, 1979), but intrinsic physiological factors may be equally important (Blower 1970; Haacker 1968; Ericson 1978).

Of particular interest to this study is the degree of plasticity a given species exhibits. Best documented for carabid beetles (Greenslade 1963; Jonasson 1973), the concept implies that the more strictly adapted to day or night activity, the greater the potential susceptibility of a species to perturbation of its biotic or abiotic environment; i.e., the more sensitive an indicator species it may be. To summarize, we will use pit-trapping data for:

a) identification of the surface-active members of the arthropod fauna and of seasonal changes in its composition;

b) identification of seasonal age structure of surface-active taxa;

c) documentation of activity patterns of selected species and their relation to extrinsic (climate, diel cycle) and intrinsic (developmental stage) factors;

d) relating population densities and trap catches, where possible, to give validity to future interpretations of variability in these ratios.

Methods: Pitfall traps containing ethylene glycol are randomly placed within each quadrat, replication and spacing between traps being determined by preliminary data analysis. Samples are gathered on a weekly schedule throughout the season.

A single trapping period encompasses 24 hours, during which one nocturnal and one diurnal sample set is obtained. Nocturnal traps are placed after sundown and collected before sunrise. Diurnal traps are placed by sunrise and collected before sundown.

As recommended by Solem and Sendstad (1978) we will take a constant route to each trap and handle it from not less than 50 cm away, to minimize "trampling disturbance". To avoid the "digging-in effect" (Joosse 1965, Joosse and Kapteyn 1968) all traps are placed one week prior to the first sampling date, their openings covered. From then on, sampling simply consists of a sequence of exchanges between empty covered traps and functional traps containing ethylene glycol.

C.   Autecological studies (field and/or laboratory) on selected arthropod species:

Autecological data provide a powerful explanatory tool for complex field population data. Specific goals of these studies will be designed to answer questions that arise from field observations and data analyses. General categories of study we foresee deal with reproduction, fecundity, feeding preference and growth rates of selected species.

D.   Litter decomposition and the decomposer fauna:

Complementary to litter input and standing crop estimates, we will set up litter bag experiments in the field, thereby

incorporating functional ecosystem criteria into our data base.

The long-term nature of this project makes it highly advisable to monitor both the biota of the forest floor and selected processes mediated by them. No two sites are ever totally alike; over periods of many years, population changes can be relatively site-specific. Should faunal successional trends be divergent in the two sites, then functional system characteristics add validative power towards distinguishing between potential ELF effects and "normal successional covariates."

Use of li ter bags allows detailed monitoring of decomposer arthropods in conjunction with decomposition processes of the substrate itself. For arthropods, organic matter and its micro-flora are a resource, the quality of which changes over time (Park 1976). The concept that arthropod communities undergo short-term successional shifts concurrent and in interaction with substrate changes has recently been emphasized (Anderson 1975; Usher and Parr 1977; Hagvar and Kjondal 1981 a,b). Results of these and similar studies indicate that:

a) the condition (decomposition phase) of litter greatly influences the composition and activity of decomposers (Seastedt and Crossley 1980; Hagvar and Kjondal 1981b; Reddy 1981); and that temporal shifts in arthropod diversity and species dominance reflect true successful changes (Hagvar and Kjondal 1981b);

b) animal succession during the decomposition process is synergistically linked to changes in the microfloral community

(Hagvar and Kjondal 1981b) but their combined effect can be altered by climatic factors (Wiegert 1974; Seastedt and Crossley 1980);

c) species that are rare in the system have the greatest value as potential indicators of disturbance effects (Abrahamsen and Thompson 1979; Hagvar and Kjondal 1981b).

Methods: At leaf-fall time, litter bags are assembled and placed in the field (10 g dry mass/bag). Sixteen bags (8 fine-mesh and 8 coarse-mesh) are retrieved from each site per sampling date. We anticipate three dates in October and November, at 2-week intervals, to track rapid mass and elemental changes in early de-composition phases. Subsequent samples (conditions permitting) are retrieved in December, monthly from April through December of the following year, and in April and May of the second year.

Litter bags are then heat-extracted to obtain faunal samples from them. Species composition of the decomposer fauna, and its changes over time, are later correlated to litter quality (decomposition phase) as well as to species composition of "natural" forest floor fauna.

Following extraction of the fauna, litter samples are further dried, weighed, and processed for nutrient analysis (P, K, Ca, Mg, Mn, Fe, Cu, Zn, Al, Mo) at Michigan State University. Samples of soil taken adjacent to litter bags in the field are subjected to the same analyses: an important quality control measure which allows correcting for soil contamination of litter bag contents.

TASK 5.4. EARTHWORMS

Earthworms are generally viewed as beneficial for soil vitality. They improve aeration and enhance mineralization by mechanical breakdown and microbial stimulation (Satchell 1967; Edwards and Lofty 1976). The type and magnitude of these effects greatly depend on the biology of the species present in a given habitat. Their occurrence and distribution in turn is regulated by the distribution of habitat characteristics (Phillipson et al 1976; Nordstrom and Rundgren 1973, 1974).

It is known that lumbricids are sensitive to electric currents (Walton 1933; Doeksen 1950; Satchell 1961), but not whether they are affected by magnetic fields. With respect to ELF electro-magnetic effects, researchers at the Wisconsin Test Facility used respiratory quotient as criterion for detecting "biological damage" to two species of lumbricids (Greenberg and Ash 1976). The test animals came from populations exposed to ELF for several years; no significant changes in metabolic rates could be demonstrated.

So far, there is no basis for selecting specific indicator criteria for detecting such effects in field populations. It is therefore imperative to document, as comprehensively as possible, lumbricid population dynamics, their place in the decomposer web, and macro- and micro-environmental factors impinging on them in undisturbed forest systems.

General sampling methods:

From snowmelt through October, samples are taken at intervals
of 3 to 4 weeks in each quadrat per site (2 samples per quadrat,
one each in hummock/depression habitat subdivisions). Each sample
subunit has a surface area of 1/16 m2, and is dug to an approximate
depth of 30-40 cm, in increments determined by the soil profile,
e.g. litter - humus - and soil in 10 cm increments.

Samples are handsorted on site, and worms and cocoons are
preserved for later identification. The soil is then returned to
the field and the sample holes are refilled. Sorting efficiencies
for worms need not be quantified (as a rule, they have been 95-98%
in our laboratory); efficiencies for cocoons may need to be checked
by washing samples through a series of sieves, if we notice that
some cocoons are tightly coated with soil and therefore easily
overlooked. Subsamples of each humus and upper soil increment are
reserved for pH and essential element analysis, while the litter
increment of each sample is dried and used for standing crop as
well as elemental analysis (10 elements, plasma spectroscopy).

A.   Species composition and population age structure:

The earthworm fauna of the northern half of North America
comprises mostly exotic European forms, and is not particularly
diverse when compared to southern regions (Reynolds 1976, 1977).
For Michigan's Upper Peninsula, virtually no records are available.
Murchie (1956) listed a total of 18 species for Michigan, but the

bulk of these records stem from Lower Peninsula collections.

For several of the lumbricids found in Ontario (Reynolds 1977) and lower Michigan (Murchie 1956) biological data are available, albeit from studies performed in Europe. Our work stresses biological and functional parameters of lumbricid populations. Many of these parameters are species-specific, and we first need to assess the composition of the fauna, the relative dominance of its members, and their seasonal patterns of recruitment and growth.

As a rule, adult reproductive specimens are used for species determination. For each species population, a seasonal age classification formula is obtained by assigning specimens to developmental stages (Reynolds 1977):

<numbers of juveniles/aclitellate adults/clitellate adults/ postclitellate adults>.

However, even dissection often does not differentiate between aclitellate and postclitellate adults, and juveniles of different species (e.g. *Aporrectodea* spp.) may be indistinguishable. Depending entirely on species composition in the definitive sites, age structure data may take the form:

<numbers of juveniles of all species/aclitellate + postclitellate adults per species/clitellate adults per species>.

B.   Population density, biomass and distribution:

The nature of the contribution of lumbricids to soil processes depends partly on inherent behavior, e.g. feeding preferences

(Zicsi 1975; Zicsi and Poboszny 1977; Satchell and Lowe 1967). The magnitude of their effects depends on population density as well as on seasonal activity patterns which are mainly regulated by climate (Graff 1969, 1971; Edwards and Lofty 1976).

Lumbricids tend to occur in associations, and density variations in the component species are determined by specific responses to environmental factors Nordstrom and Rundgren 1974). In forests, patchy distribution of resources can result in complex patterns of horizontal distribution of different members of an association (Phillipson et al 1976). In addition, vertical distribution and activity vary with seasonal temperature and moisture cycles (Satchel 1967; Gerard 1967).

Density and biomass are clearly interrelated, and both will change through time with changing size structure of a population. These three attributes, monitored seasonally, accurately depict lumbricid population dynamics, especially if combined with autecological data. Population distribution in space, notoriously variable (Satchell 1971), can best be interpreted if the distribution of pertinent environmental variables is monitored, as we propose to do; through appropriate analyses, e.g. multiple regression, we thus relate site-specific population parameters and selected ecosystem attributes.

By identifying and categorizing lumbricids obtained through the season, density estimates and spatial distribution will be arrived at as a matter of course.

Biomass estimates are much more controversial and subject to error. Lumbricid live weight depends on water content, i.e. physiolgoical state (French et al 1957), and may therefore differ seasonally within a given size class. Length of individuals can be used for estimating weight, and may give more reliable results than direct live weight (Satchell 1971) because gut content may introduce an error of about 20% (Bouche 1967).

Abrahamsen (1973) has shown that very high coefficients (0.95 to 0.98) can be obtained for correlation between length of preserved individuals and live weight, providing the relationship is quantified for each species and major size class within species. We will therefore use specimens obtained in definitive sites to standardize techniques and analyze them for the most accurate means of obtaining biomass estimates, using: live weight, full and empty gut; body length after killing in alcohol and 48 hrs in formalin; and dry weight from the same specimens after drying to constant weight.

C.   Reproductive Biology:

Most lumbricids are potentially active and breeding year-round, but adverse conditions will cause them to move into deeper soil strata and/or to enter diapause (Evans and Guild 1947; Gerard 1967; Grant 1956). Especially in northern climates, many species hibernate or estivate in tightly coiled resting stages, thereby interrupting breeding as well as growth (Nordstrom 1976; Rundgren

1977). Cocoon production thus becomes seasonal, but temperature (Evans and Guild 1948; Gerard 1967) and soil moisture (Graff 1953) influence incubation time. Cocoons may overwinter, so that a season's young can partially be the result of the previous fall's reproductive effort.

A few estimates of cocoon densities in the field are available (e.g. Gerard 1967). More frequently, patterns of juvenile emergence are used as indicators of reproductive activity (Rundgren 1977; Nordstrom 1976). These data quantify population recruitment; their interpretation often relies on laboratory-derived estimates of cocoon development time, which is temperature-dependent, and fecundity, which is species-specific (Evans and Guild 1948; Reynolds 1973).

Like other reproductive parameters, weight and content of cocoons may vary seasonally (Watanabe and Tsukamoto 1976), although this variability probably does not apply to lumbricids in general. Cocoon weight may be a useful measure, but taken alone is not an indicator of its contents. In Allolobophora rosea (Savigny), for instance, cocoon weight indicates developmental state of the adults that produced them, due to differing weights of the cases, not differing juvenile weights (Phillipson and Bolton 1977).

In summary, documentation of reproductive biology of lumbricids is best achieved by combining seasonal cocoon densities (empty and full) in the field, juvenile numbers per cocoon, emergence patterns of young, and proportion of adults in

reproductive condition. If necessary, observation of cocoon production and development under near-field conditions can be used to supplement information on incubation time, emergence weight, and climatic regulation of fecundity for each species.

Specific methods to be used for quantifying reproductive parameters will be dictated by lumbricid species composition in the definitive sites. Soil-dwelling species, for instance, typically produce few cocoons per adult and year (Evans and Guild 1940); in combination with low population density, very few cocoons may be recovered; as a result, data may have to be pooled over more than one sampling period in order to increase replication. Essentially, data obtained from handsorted samples during the first full season will determine specific future goals and methods. Where necessary, field data on reproductive biology will then be supplemented by controlled autecological observations: e.g., rearing of subadults to maturity to ascertain reproductive method (amphimixis, partheongenesis); rearing of adults to obtain cocoons of known species, to be used a reference for interpretation of field data; fecundity and viability observations under field and/or constant laboratory conditions.

D. Ecological classification of lumbricid associations:

For large-scale distribution surveys of lumbricids, Julin's (1949) ecological classification system appears useful; e.g.;

Reynolds et al (1974) used it for a comparison between the earthworm fauna of Tennessee and that of Ontario. For relatively local studies, however, Bouche's (1971, 1977) system is much more applicable. Its species-specific criteria ar more detailed as well as being oriented toward soil processes. The system utilizes a tri-polar gradient, the poles being:

- "endogees", species that live in, and feed on, soil;

- "ancieques", which live in deep burrows and feed on the surface;

- "Epigees", species that live in an feed on litter, bark and other non-soil habitats.

Intermediate forms are placed along the gradients between these poles. The system uses discrete parameters; their quantification is the responsibility of sensitive field and laboratory monitoring programs.

Each of Bouche's categories is recognized by an assemblage of physiological, behavioral and anatomical characteristics. A generalized, abbreviated list of these criteria, adapted from Bouche (1977) is given below:

a.   spatial distribution (habitat preference);

b.   feeding preference (soil and/or litter);

c.   physiology (reproduction, diapause);

d.   growth (seasonal age structure);

e.   activity patterns (mobility; casting habits);

f.   morphology (internal, external).

Essentially they describe species-specific adaptations; taken together, they form the basis as well as the explanation for the functional place of a given species in its habitat and in the ecosystem as a whole.

Once definitive sites are established, their lumbricid associations will be characterized according to these criteria; biological and physiology criteria not quantified through the objectives discussed earlier will be obtained as needed. Ecological classification is not merely an exercise in data synthesis, however. It has value to this project because it provides a framework against which to measure its conceptual as well as actual progress. Lack of, or error in, specific parameters becomes readily apparent, so that a well-quantified data base of species-specific, interdependent parameters can be rapidly accumulated.

RESULTS AND ACCOMPLISHMENTS: JULY-NOVEMBER 1982

THE FIELD FACILITIES

A house in Channing (Dickinson County) and a nearby barn were leased in late July, providing living quarters as well as laboratory space for: extraction of samples; processing of faunal samples prior to storage; processing of litter and soil samples for chemical analyses; microscopic work; and storage of field and laboratory supplies and equipment.

Three separate banks of Tullgren-type heat extractors were constructed, yielding a total extraction capacity of 60 samples. The number of banks, and therefore the number of samples that can be extracted simultaneously, will be doubled in the first month of the 1983 field season.

SITE SELECTION

Constrained by not knowing final antenna location and by the need to begin sampling as soon as possible, we initially selected two potential sites in cooperation with the Department of Natural Resources (Fig. 3). The main site, a maple-basswood stand on Turner Rd., was used intensively for faunal sampling. The secondary site (Silver Lake Rd.), with a greater percentage of poplar than Turner Rd., was used to quantify certain aspects of pit-trapping methodology, and will not be sampled in the future.

Later in the season, the area was again canvassed for potential study sites. Six additional sites, all maple-basswood stands in similar stages of maturity, were located (Fig. 3). Final selection of paired sites in 1983 will be contingent upon definitive antenna location, as well as on measurement of existing levels of electromagnetic radiation (e.g. due to power lines).

SAMPLING PROGRAM AND PRELIMINARY RESULTS

Our major goal was to obtain a collection of specimens representative of the soil-litter fauna of maple-dominated
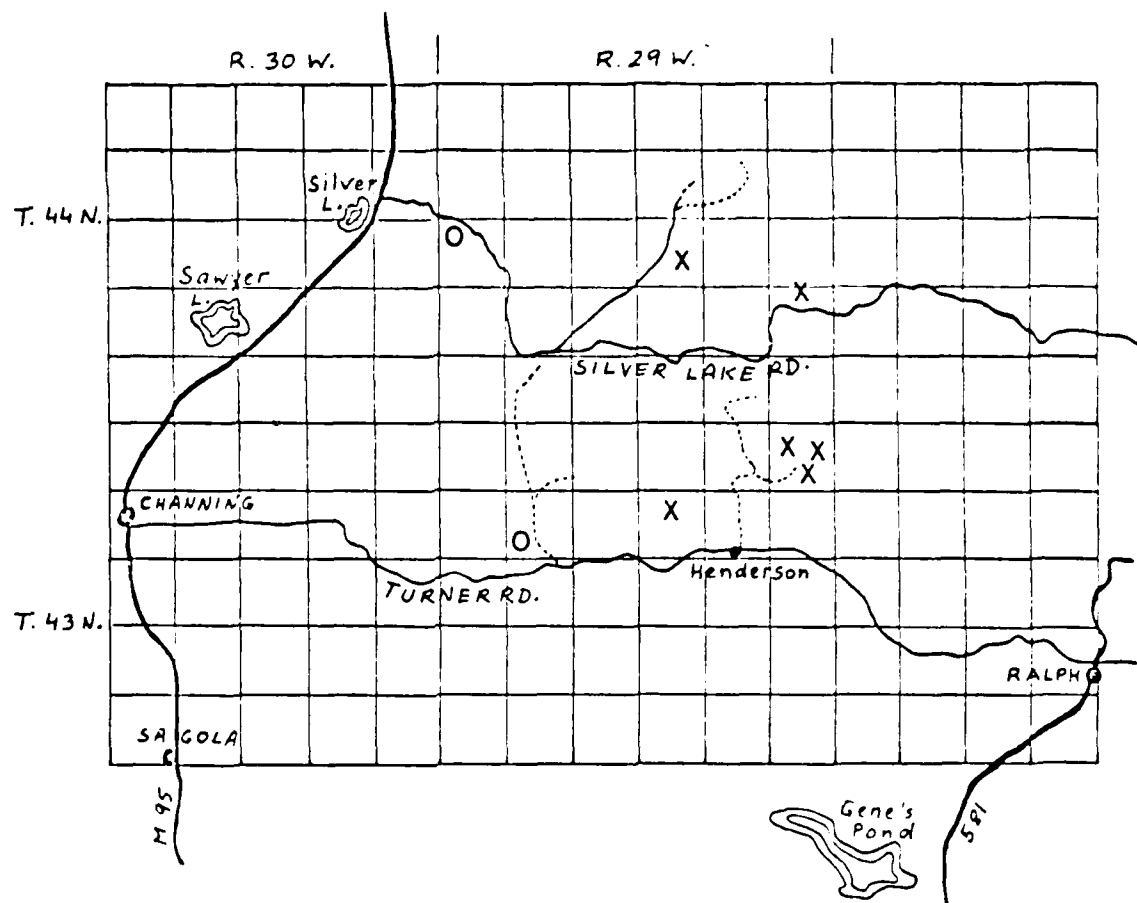
O Turner Rd. and Silver Lake Rd. sites sampled 1982

X Additional maple-basswood sites available for final
selection of paired plots in 1983

Figure 3. Map of study sites surveyed in 1982; all
are in Ford River State Forest, Dickinson County.

forest in the ELF antenna area. Sample totals for the 1982 season are summarized in Table 1. The faunal material obtained from them is sorted to species in the case of lumbricids; arthropods are initially sorted to order or family level; further breakdown to genus or species level is accomplished during winter and spring.

More detailed comments, and preliminary results where available are given below.

Table 1. Summary totals of arthropod and lumbricid sampling programs, early August to November 1982.

SAMPLE TYPE	REPLIC./DATE	NO. SAMPLING	TOTAL SAMPLES
Earthworm 1/16 m^2 handsorting	20 (2 subsam. each)	3	60
Soil cores heat extrac.	20	5	100
Litter 1/16 m^2 heat extrac.	20	5	100
Distance series	240 (48 x 5 days)	4	960
Diel series	24 (12 + 12)	8	192
Undergrowth series	20	5	100
		TOTAL	1512

A.   Earthworms (Turner Rd. site):

Samples (25 x 25 cm to an average depth of 30 cm) were taken
at intervals of two weeks and handsorted.   Two localities were
selected (N = 10 samples in each): small-scale hummocks and litter-
rich depressions, for the purpose of assessing habitat hetero-
geneity as a source of variation in lumbricid distribution.   Such
topographical heterogeneity is a characteristic common to all
potential study sites examined.

Check for presence of deep burrowing forms: following removal
of regular samples by digging, formalin extraction was used on the
bottom of the sample holes.   The procedure was repeated on two
dates, for a total of 30 samples.   The technique, used specifically
for burrowing species such as Lumbricus terrestris, gave clear
negative results; i.e., deep-living species were not present at the
site.

In this preliminary sampling program, the litter layer per se
of each $1/16$ m^2 sample was used to heat-extract arthropods.   In
the following, lumbricid density estimates therefore do not include
the relatively small segment of the population inhabiting leaf
litter.

Preliminary results:

The lumbricid association of the maple-dominated Turner Rd.
site consists of five species, all natives of Palearctis, discussed
below in approximate order of decreasing density:

Dendrobaena octaedra (Savigny): the species is most common in
the upper organic horizons, and is said to feed selectively on
litter (Reynolds 1977); parthenogenetic morphs are numerous (Gates

1974).

Octolasion tyrtaeum (Savigny): a relatively large-bodied, obligatorily parthenogenetic species, for which varied and contradictory habitat preferences have been reported (Causey 1952; Eaton 1942).

Lumbricus castaneus (Savigny): an amphimictic species (Reynolds 1974). According to Reynolds (1977), individuals may retreat deep into the soil under adverse conditions (presumably drought); no evidence of such behavior was found under the study site conditions during the limited 1982 field season.

Aporrectodea tuberculata (Eisen): a large-bodied, amphimictic species which probably breeds in spring and autumn (Gates 1972).

Eisenia rosea (Savigny): a parthenogenetic species (Evans and Guild 1948) which appears to be the numerically least important component of the lumbricid association at the study site.

Species-specific phenological data are not yet compiled, but analysis of total numbers per sample (Table 2) shows that the site harbors a relatively dense lumbricid fauna when compared to beech, oak and mixed woods in Europe (Zajonc 1970; Satchell 1967; Reynoldson 1955) and North America (Reynolds 1969, 1972).

Table 2. Earthworm population, Turner Rd. site: preliminary
analysis of data obtained by handsorting (not included: specimens
extracted from the litter layer of each sample).
D = samples in depressions (n=10); H = samples from hummocks
(n=10); sample surface area = 1/16 m^2.

	DATE AND LOCALITY					
	August 9-12		August 25-28		September 7-10	
	D	H	D	H	D	H
mean worms/s.	20.9	23.4	13.3	14.2	17.8	16.5
$\pm$ SE	4.6	4.2	2.8	1.9	4.0	2.4
(SE as % of $\bar{x}$)	(22.0)	(17.9)	(21.1)	(13.4)	(22.5)	(14.5)
Ratio worms:cocoons	3.7:1	26.0:1	2.1:1	3.2:1	3.2:1	13.8:1
% of worms						
in H horiz.	68	84	52	62	90	79
in A_1	25	15	36	20	7	16
in A_2	7	1	12	18	3	5
% of cocoons						
in H horiz.	77	67	87	93	95	83
in A_1**	20	33	13	7	4	17
in A_2**	3	0	0	0	1	0
	D+H		D+H		D+H	
density estim. per m^2 $\pm$ SE	354 $\pm$ 50		220 $\pm$ 27		274 $\pm$ 37	

**) The terms A_1 and A_2 are used loosely here, since there was
variation of horizon characteristics between samples; essentially,
A_1 samples were taken to an average -10 cm below the H layer, and
A_2 samples to an average -10 to -20 cm below H.

The bulk of the population inhabited the humus layer, which is
also the predominant site of cocoon deposition. Hummock samples
occasionally contained low numbers of cocoons (Table 2), but
possible differential cocoon distribution may be neither consistent
nor significant and will be investigated further.

Mean density of worms per sample did not differ significantly between D and H localities (Table 2), in spite of the fact that average depth of the humus horizon in depressions was double that on hummocks.

Sample variability was somewhat less within hummock samples than depression samples. Expressed as percent of means/sample, standard errors can be taken as a measure of replication sufficiency: for lumbricids, sampling errors of 20% of the mean may be considered "reasonable" (Satchell 1971), in view of the man-hours needed to perform the work. Based on our 1982 data, 20 replicate samples per date should keep samling error at 15-20% of the mean. Replication will, however, need to be validated again for the site-specific lumbricid associations in definitive Test and Control sites.

B. Arthropods (Turner Rd. and Silver Lake sites):

As evident from Table 1, area-specific (quantitative) soil and litter arthropod samples were obtained by Tullgren extraction; these sampling programs were discontinued at various times during October 1982. Sorting and identification of this material will not be completed until late spring 1983; the arthropod community can then be described and quantified.

Three pit-trapping programs were followed during 1982:

a) "Distance series" (Silver Lake site):

To assess the effects of distance between traps (potential over-exploitation of the sampling area), four transects containing 12 traps each were established. They differed with respect to intervals between traps: 0.5 m in transect 1, 1.0 m in transect 2,

2.0 m in transect 3, and 4.0 m in transect 4. Empty traps were left in place for 14 days, then were activated with ethylene glycol and collected every 24 hours for five consecutive days (on purpose, this was a more intensive trapping schedule than we would normally use). Four (5-day) time series of 12 replicates per transect were thus obtained.

b) "Diel series" (Turner Rd. site):

To obtain data on species composition of diurnal and nocturnal arthropod groups, traps were activated just after dusk and collected the following dawn (nocturnal samples) and dusk (diurnal sample). The program was followed at weekly intervals for a total of 8 diel series.

c) "Undergrowth series" (Turner Rd. site):

The purpose of the program was to obtain a qualitative estimate of within-site faunal variability related to vegetation density and distribution; i.e. to assess the potential influence (source of variability) of a shrub-sapling understory under sparse canopy on the composition of the surface-active fauna. Every two weeks, 20 replicate traps were activated and collected after a single 24 hour period.

Comparative analysis of data obtained through b) and c) will aid in determining the experimental design of quadrat subdivisions in definitive sites; it will also apprise us as to the number and extent of sampling programs needed to obtain as complete a species list as possible for the systems (sites) as a whole.

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Roland L. Fischer, Principal Investigator
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Authorized Subcontractor and Releasing Authority

Table of Contents

ABSTRACT

Some 40 species of megachilid bees, representing eight genera, in complexes which are indigenous to North America are known to occur in the Upper Peninsula of Michigan. Each of these species is restricted in their ecological niche requirements for nesting sites; plants from which they obtain pollen to be used as provisions for growth and development of the larval stage; flowering plants from which nectar is extracted for subsistence of the adult or as admixtures with pollen; plant or mineral materials for construction of the nest; innate behavioral differences in securing the above materials and method for storing and/or manipulation at the nest site; a complement of predators and parasites; and an annual phenological sequence which is generally coincident with flowering activity of the plants to which they are restricted.

Current research efforts aimed at integrating the various facets of megachilid biology are underway. These include an assessment of plant species known to be of use to the bees which are indigenous to the study area, the species complex of the megachilids of the area, and a literature survey on the biology of the species of bees of the area and preparation of such available data for computerization and later direct comparison with field data obtained in this current project.

4

SUMMARY

Approximately 40 different kinds of megachilid bees, known commonly as leafcutter bees, are known to occur in the Upper Peninsula of Michigan. Each construct a nest consisting of several cells in some kind of hole. Cells are separated from each other by leaf parts, petals or flowers, or some other plant parts. Each cell is provisioned with pollen and an egg laid upon the pollen mass. The immature stage, the larva, thence feeds upon the provisioned pollen mass until maturity and will emerge the following season.

The intricacies involved in securing pollen and its storage, the range in kinds of plants used, and the instinctive behavioral mechanisms varies with each of the kinds of bees. Aspects of these biological facets will be studied in this project. Sites for study will be maintained some distance from the ELF antennal system as a control or check, others will be placed in the immediate vicinity of the system. Climatic factors which may alter a behavior pattern of a bee will also be obtained. Any deviation or difference in the biological pattern of the various kinds of leafcutter bees under study between the control and research study areas may thus be ascertained.

RATIONALE

The common honeybee, _Apis_ _mellifera_ Linnaeus, an imported species from Europe is thought to be the single most important pollinator of North American flowering plants. This is perhaps true of some species of flowering plants endemic to the Holarctic Region, or of differing species in North America which are closely related to others in Europe, to which the honeybee has adapted by coevolution through time for the collection of pollen and nectar. Our native North American bees, of which there are about 3500 known species, have likewise coevolved through millenia of time with the native North American flowering plants. The adaptations of the bees for collection of pollen are unique and correlate with peculiarities of floral structure with native plants. These adaptations have evolved so distinctly that some native bees are known to restrict visitation to a single species of flower, a group of closely related species, or sometimes a group of closely related genera for which the term oligolectic is applied. The honeybee is a polylectic species, visiting flowers over a wide range of species whose nectar and/or pollen are readily available.

Overwintering studies on the honeybee would be excessively difficult to accomplish because of the length of the winter period (days below $50^{o}F.$), extensive snow cover from mid-October through April, and excessive energy requirements for the bees to overwinter. The long period of temperatures below operable conditions for flight of the honeybee combined with snow cover negates the possibility for a cleansing flight of the honeybee during late winter period -- the net result being the death of the colony!

The experimental site being situated in the heart of the contiguous Michigamme, Escanaba River and Ford River State Forests has not been disturbed by man's agricultural efforts as much as other areas of the U.P.

where agriculture has continued over a period of years. Forage areas for
honeybees have tended to be associated with agricultural areas in the U.P.
-- near acreages of legumes and other cropping systems which have disturbed
areas adjacent which has lead to a plant succession and species of plants
more conducive for honeybees to use as forage.

Apiarists in the U.P. have long been cognizant of these facts. Colonies
are placed adjacent to agricultural lands where abundant forage potentials
may be found, are killed in late fall, and the entire honey supply is har-
vested. From an economic aspect it is cheaper to begin the following season
with purchased nukes from southern states than it is to attempt to overwinter
the colonies and take the chance of them being dead when spring arrives.

For reasons outlined above, specifically: (1) that the honeybee is not
native to North America and has not co-evolved with the native North American
plants and as such are not the primary pollination agent of many plants
found in the experimental site; (2) that the native bees have co-evolved
with the North American plants and are thus far more efficient in their
pollinating activities; many of them being oligolectic; (3) that forage
areas for honeybees in the experimental area are limited; and (4) that
overwintering studies with the honeybee would be most difficult, that
research efforts will be restricted to the basic biology of our native
megachilid bees.

SCIENTIFIC APPROACH

I. GENERAL BIOLOGY OF TRAP NESTED BEE

The native bees are generally solitary in respect to their nesting
activities. The mated female may construct her own hole to make a nest
by burrowing into the soil or a pithy plant stem, or make use of a previously
made hole in wood such as an emergence hole of a beetle. Researchers have
made use of this need for a hole in wood by drilling holes of varying
diameters in wood and setting the blocks of wood out in nature in pro-
pitious places. Other techniques have included soda straws, hollow thatch,
or pithy stems. The method is generally referred to as "trap-nesting."

The selection of type of nesting material and whether the hole is
previously present is dependent upon the species of bee. After selection
of a proper nesting site the female collects pollen or a mixture of pollen
and nectar for deposition in the tunnel. After many trips, she lays an
egg upon the mass of provisions and partitions off the area, a cell, leaving
enough room for the developing larva to increase in size (see Figure 1).
The cell partition may be composed of resin, sand, soil, cut-out portions
of leaves, masticated leaves, plant down or in some cases mixtures of the
aforementioned materials -- again dependent upon the species. Several
cells may be formed in a linear arrangement along the length of the nest.
A vestibule cell is sometimes formed between the last cell and the nest
cap. After completion of the nest cap the female abandons the nest and
may seek out another suitable hole in which to begin another nest.
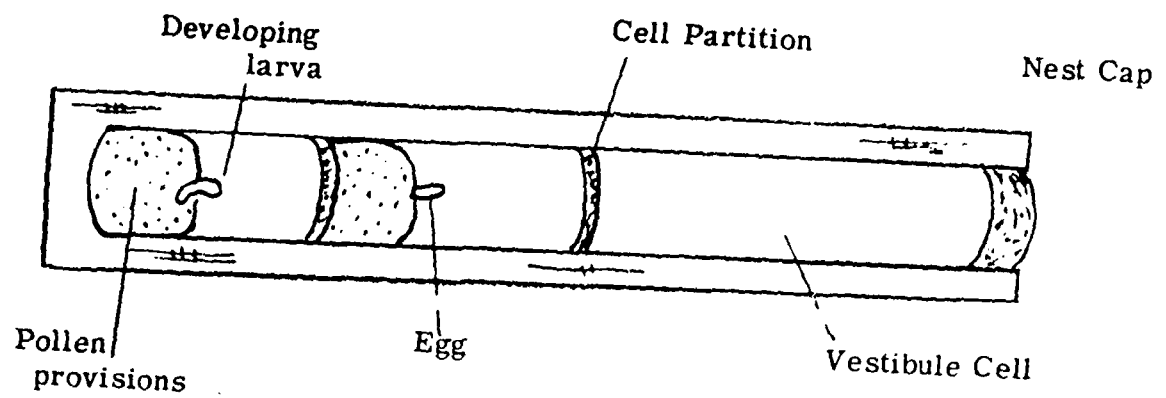
Figure 1.   Longitudinal section (schematic)   of a
             megachilid bee trap nest illustrating terminology used in text.

The process of nest construction may be repeated several times during a summer season. Environmental parameters play an important role in the length of time for any of the elucidated gathering procedures. In the case of oligolectic bees the life span of the bee is totally dependent upon the phenology of the plant the bee is restricted to and may thus have a very short life span and produce a single nest of very few cells.

II. TRAP NESTING METHODOLOGY

In the study site area five genera of megachilid bees are known to occur which are commonly found in trap nests. These include Dianthidium, Osmia, Hoplitis, Megachile, and Heriades – represented by some twenty species. Some of the species of Osmia and Megachile commonly occur in the area so there is no doubt that large numbers of nests could be obtained in the study.

Nesting blocks of select white pine (3 X 3 X 6 in) will be bound together in units of nine. Holes will be drilled lengthwise of the trap nest and arranged in the pattern shown in Figure 2. The binding in units has been shown by the principal investigator to allow for easy manipulative handling, observation, and recording of data.
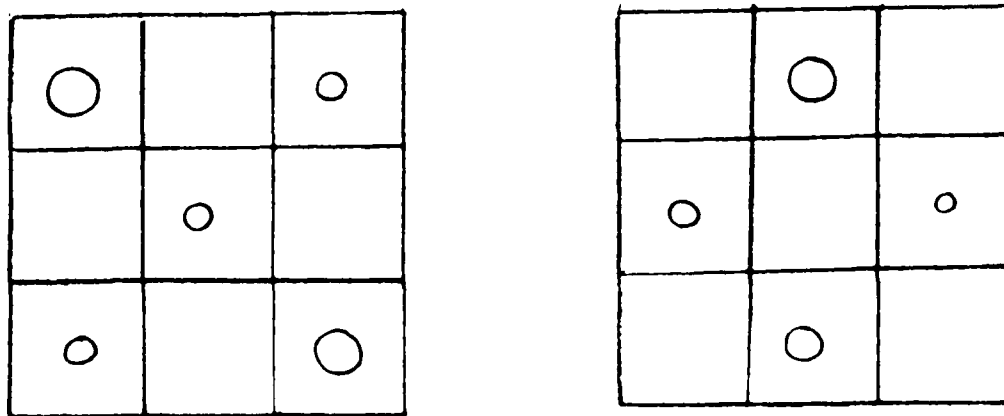
Figure 2. Schematic diagram illustrating opposing end views and random arrangement of varying size bores.

The assembled units are then placed in a randomized pattern on shelves of hutches. Construction of a 3 shelved hutch is shown in Figure 3. The randomized pattern eliminates any problems with homing and the return of the bee to the proper nest. Placement of the hutch in the environment is a critical factor - semi-open areas with abundant mature flowering plants, other plants used by the bee in nest construction, and availability of other nesting materials being the more important.
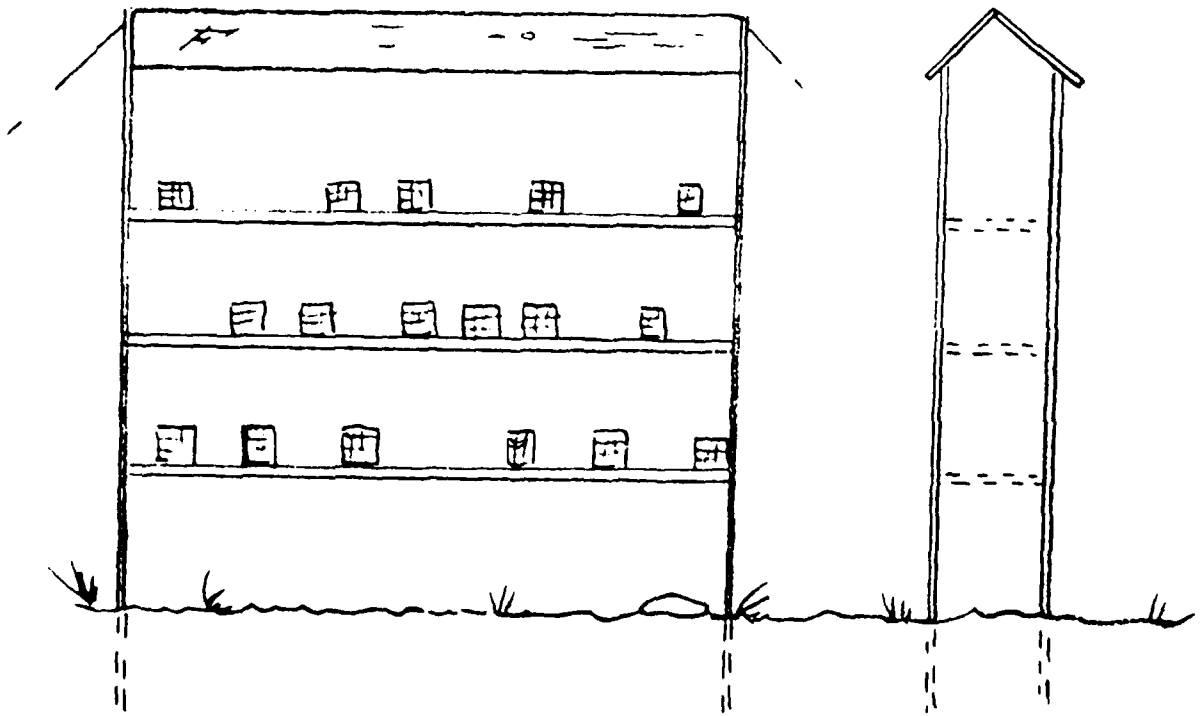
Figure 3. Side and end views of hutch construction and placement of nesting blocks.

III.   OBSERVATIONS ON NESTING ACTIVITY

Data will be obtained as to date and time at which a nest is founded and eventually completed. Daily records will be kept on the progress in the nesting cycle for each nest.

A selected number of nests will be monitored by the investigative team by visual observation to generate the following types of data:

1.   Numbers of trips required to provision with pollen for each cell.

2.   Intervals of time necessary to unload and arrange pollen within each cell.

3.   Numbers of trips to secure building materials for partitions.

4.   Intervals of time between trips in 3 to construct partitions.

5.   Time required for egg deposition for each cell.

6.   Intervals of time between trips in 6 above to construct the nest cap.

7.   Numbers of trips to secure materials for capping the nest.

With the founding of each nest the female bee will be marked in such a manner that she will be recognized either at the nest site or in the field at some distance from the nest. Records will be kept on flight range, flowers visited for nectar, flowers visited for pollen (if different), and an attempt will be made to ascertain the source and activity mechanisms involved in the nest partition materials and capping materials. Marking studies will also allow for records to be maintained on nest supersedure and usurpation.

Certain selected nests will be opened immediately upon completion to ascertain the time period of eclosion of the egg after deposition, the developmental rate of the developing larvae, the time intervals between molts, survival rate, and the stage of development at the close of the season.

With the approach of fall and cessation of field activity the nests will be stored in the locations in which they were made -- the only protection being afforded would be some kind of screening device to prevent marauders such as bears, blue jays, flickers, rodents and other mammals from destroying them for food. The overwintering nests will be split open in late April or early May of the following year to ascertain the following:

1. The number of cells per nest

2. Bore size of tunnel

3. Stage and condition of the immature within each cell

4. Length of each of the partitions

5. Construction materials of partitions

6. Presence of vestibule cell -- size of same

7. Anomalies in nest architecture

8. Length of nest cap

9. Incidence of parasitoids - predators.

Opened nests will be stored in test tubes, closed with nylon gauze, and returned to their original nesting sites. They will be opened and observed daily as to progress toward adulthood, time and duration of pupation, each individual cell will be sexed, and upon emergence as an adult the animal will be marked and released at the nest site from which it originated.

Selected specimens from each of the nests will be retained for later taxonomic verification as to the species and mounted in the usual manner. In addition to the usual date-locality label additional labeling would include a distinctive colored label noting that it was part of project ELF and include a nest number, cell number, emergence date, and plot locality. These specimens will be placed in the Entomology Museum of Michigan State University for permanent deposition as voucher specimens.

IV. ENVIRONMENTAL INFLUENCES

Of the 200 papers that have previously been written on the biology of North American trap nested bees and biology of native bees in general there are but a few general remarks which allude to parameters within the environment which directly impinge upon the behavior of the bees. The principal investigator has found with his work that many unanswered questions relate to environmental factors. Thus, both from the standpoint of the project and also to delimit some of these parameters in relation to native bee biology the following will be monitored:

1. Ambient air temperature to ascertain flight behavior, optimum temperatures for flight to occur, whether higher mid-afternoon temperatures affect rates of gathering of nesting materials, etc.

2. Relative humidity to note similar affects as in 1.

3. Precipitation will definitely impede the movement of the native bees. Knowing time intervals when rain occurs in an area it would be possible to reconstruct time intervals in nest construction events. It is also not known as to the severity of storms and how much affect is produced.

4. Barometric pressure -- the principal investigator in his work on Megachile pugnata Say has noted several instances in which the female bees returned to the nest blocks more or less en masse and remained in their nests in a night time attitude. At those times the sun was shining brightly, but within the next hour a storm approached. Are these animals capable of detecting changes in atmospheric pressures? With a monitoring mechanism and visual records we might have some clues as to these strange behavior patterns.

5. Solar radiation -- native bees are very sensitive to changes in light, especially related to flight activity. It is thus necessary to define these limits more astutely.

It is considered imperative that the above listed natural ambient environmental influences be monitored for this proposal. Any of the above listed parameters could have a direct influence upon the behavior mechanism of the bee resulting in marked differences in nest architecture. By correlating environmental factors with nest architecture or events in the biology of the native bee in a statistical method it could be possible to pinpoint the causal agent.

V. AMBIENT MONITORING

Introduction

During the initial years of this investigation, it is desired to establish a data base on the unperturbed natural biological phenomena and processes occuring in the study area. Only by comparison with such a reference data base can ecological responses to potentially disturbing influences be detected and evaluated. A discussion follows of the methodology for the acquisition of ambient environmental measurements to establish such a reference data base, and later to provide initial environmental data for analytical correlation with the studied ecosystem's reactions to ELF influences.

General Approach

For economy and reliability, it is proposed to automatically (electronically) monitor and record ambient environmental parameters amenable to such sensing techniques. Six field instrumentation modules (Model Ti-5X, custom configured by the Instrumentation Division of Eco-Tech, Inc.) will be purchased for automatic monitoring. These units will be assembled in ruggedized, environmentally "hard" enclosures for extended field use. Due to the nature of the Elf experiments, all electronic sensing equipment has been selected to be battery operated.

Further, the instrumentation clusters will be configured to be not only immune to ELF fields, but to exhibit negligible EM radiation from instrumentation equipment and cabling.

Ambient environmental data acquired via the monitoring systems proposed in this document is designed to be compatible with our data management plan. That plan provides for a custom-configured and programmed microcomputer to be operated near the field study area for data entry, consolidation, preview, and preliminary analysis.

The relevant ambient environmental conditions proposed to be monitored and recorded via the automatic Model TI-5X instrumentation modules during the period of investigation are listed in Table 1, below.

Table 1. Automatically sampled ambient environmental parameters

	Parameter	Sensor	Frequency
1)	Ambient Air Temp	Thermistor	30 mins.
2)	Relative Humidity	Elec. sensor	30 mins.
3)	Solar Radiation	Pyranometer	30 mins.
4)	Rainfall	Tipping bucket	Time of tip
5)	Barometric Pressure	Solid State sensor	30 mins.

Date Acquisition System

It is planned to monitor air temperature and relative humidity, solar radiation, precipitation and the barometric pressure in an open

area near the experimental nesting locations. At each sampled location, the five parameters will be monitored and logged by a TI-5X instrumentation module which will contain two separate two-channel and one single channel solid state data loggers (Omnitronics Datapods). These logging devices will be installed in a weatherproof enclosure installed below ground level (to discourage vandalism). The logging systems are each capable of storing 1023 sets of readings on a removable data storage module (EPROM), allowing more than a month's readings taken every 30 mintues. The systems will operate on internal batteries (8AA cells) for at least four continuous months. The site microcomputer will be outfitted with a data storage module reader for data transfer. The data storage modules are erasable and reusable indefinitely.

Ambient Air Temperature and Relative Humidity

Implementation - We plan to monitor air temperature and relative humidity using a General Eastern R.H. and Temperature Transmitter Model 455. Eco-Tech's experience with several similar R.H.temp instruments has shown this particular device to be reliable, accurate, and compatible with the proposed lo-power consumption, battery-operated scheme. The sensors are to be installed on a USWS "cotton-region type" enclosure with double roof, louvered sides and slatted bottom.

Calibration - the temperature sensing thermistor assembly will be compared to a laboratory quality mercury thermometer mounted near the sensing assembly; any deviation from the reference thermometer will be recorded and such information will be used to correct the logged temperatures. The R. H. probe reading will be compared to a sling psychrometer reading weekly. If any significant difference is noted, the R. H. probe will be recalibrated via the saturated salt vapor pressure bath method, using potassium sulfide for a high (>90%) reference and lithium chloride for a low (<18%) reference. This calibration check will be performed bi-weekly.

Solar Radiation

Implementation - Incident solar energy will be sensed by a Li-Cor Model LI-200S pyranometer. The Datapod logging device will sample the sensor value every 5 minutes and the resulting average logged every half hour.

Calibration - logged values will be compared to other readings nearby. If a significant deviation is noted through weekly preliminary data analyses, the subcontractor will be notified for corrective action.

Precipitation

Implementation - Rainfall will be monitored by a Weathertronics Model 6010 tipping bucket rain gauge. This device produces a momentary closure of a mercury switch for each 0.25 mm of accumulated rainfall. Each switch closure triggers the date logging device to store the time (to the nearest minute), thereby recording rate as well as total rainfall. During the winter months, data for precipitation and accumulation will be obtained from the U.S.A.F. weather station at K.I. Sawyer AFB near Guinn, Michigan.

Calibration – the tipping bucket rain gauge will be calibrated weekly by pouring a pre-measured volume of water through the sensor mechanism and noting the logged rate. Any erroneous readings will be immediately reported to the instrumentation subcontractor for corrective action.

Barometric Pressure

Implementation – The local barometric pressure will be monitored by a Weathertronics Model 7115 solid state barometer. The analog pressure sensor will be mounted in the same instrument shelter used for air temperature and relative humidity. The sensor output will be sampled every five minutes. Every half hour, the preceeding six values will be averaged and logged.

Calibration – The barometric sensor will be compared weekly against a portable precision survey altimeter calibrated in millibars; this portable device will be frequently compared and calibrated against a mercurial barometer. Any deviation from the reference barometric reading will be recorded and such information will be used to correct the logged pressure.

VI   EXPERIMENTAL DESIGN

Units of nesting blocks will be placed randomly on the shelves of 18 hutches as shown in Figure 5. This will approximate some 2600 potential nesting sites for the native bees. From this potential we would expect approximately 1000 nests completed during a full season of activity -- more than ample to obtain data on the nesting biology of several species of native bees.

The nesting blocks will be distributed in four distinct sites:

1. An area immediately below the ELF Communications Systems Cables

2. An area immediately above the below ground Systems

3. An area approximating one mile distance from 1 and 2 above

4. Two areas (the controls) approximating some 10 miles distance
    from the Cable System.
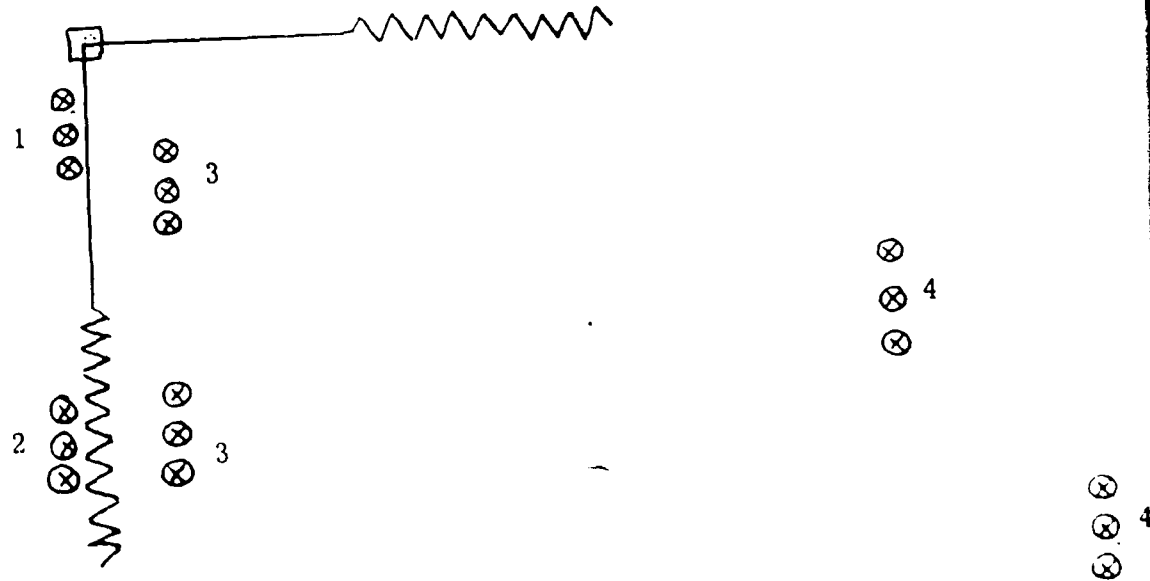
Figure 5.   Diagramatic scheme for placement of hutches:
             (1)   immediately below ELF Communications Systems Cables;
             (2)   below ground;
             (3)   one mile distace from (1);
             (4)   two sets,10 miles from cable system (controls).

Any external pressure upon the population of a native bee, whether a
dramatic climatic change or other naturally occurring phenomenon or some
man-made force, could seriously alter the ethological pattern of nest
construction and provisioning or the synchronous development of an obigol-
ectic bee and a flowering plant. This could result in a subsequent dis-
ruption in the population of both the native bee and plant. Statistical
correlations may be made within a species of any of the above mentioned
ethological nesting data, any or all (or in several combinations) of the

ecological factors as obtained by the ecological monitoring program,
and compared with the four test sites of the Cable System and controls.
Alterations in the behavior mechanisms of the native bees, if any,
brought about by the electric and/or magnetic fields associated with
the proposed Project Seafarer should become apparent by these cor-
related statistical studies. Perhaps, as important, the design and
approach in the above experiments will enhance our fundamental
knowledge through publication of results on the biology of some
pollinating insects and some of the ecological parameters which
impinge upon them.

SUMMARY OF ACCOMPLISHMENTS

When the budget for the megachilid bee project was initially
predicated it was anticipated that we would be in the field and set-
up work during the second week of June, 1982. For numerous reasons
it was soon apparent that we could not be in the field much before
the end of July. At this time the nesting season for the genus
Osmia, a genus which contains approximately 10-15 species in the
Upper Penninsula, had terminated. Likewise, the normal nesting per-
iod for members of the genus _Megachile_, comprising another 10-15
species, was nearly half completed. It was thus apparent that aspir-
ations for a full season of research effort on bee activity were nil.

Also, in the limited time available materials could
not be ordered, constructed, and assembled to make the hutches and nest-
ing blocks for placement in the field situation.

Research efforts were thus abruptly changed to take advantage of this
time lag to better prepare the project personnel with background informa-
tion in preparation for an early start-up time for the 1983 season. These
may be summarized as follows:

1. Assessment of sites for trap-nests.

2. Species of megachilids in the Upper Peninsula.

3. Plants used by megachilids.

4. Previous literature on megachilid biology.

5. Set-up of computer programs.

1. Assessment of Sites for Trap-Nests

It should be apparent that the restrictions brought about by pheno-
logical events, the obligate relationships of the several species of
megachilid bees with plants for nesting materials and food, and differences
in the selection of sites for nesting make for a complex of entities that
must be met if they are to abound in any area. The necessity for careful
analysis of a potential site for placement of trap-nests is thus paramount.
Considerable time has been expended in August and September in this act-
ivity. Some 14 potential sites have been designated -- some of which will
hopefully be coincident with the eventual placement of the cable system.
As important, many areas on the survey were eliminated from future plans
because they did not meet some of the criteria listed above. Control plots
have been selected at both Sagola and Channing, Michigan, some 10 miles

distant from the originally proposed installation. These check plots will
be implemented as soon as weather permits in the 1984 season. Placement
of other hutches in the experimental areas will depend upon eventual dis-
position of the cable network.

2. Species of Megachilids in the Upper Peninsula

Because of the complexities in the determination to species level of
the 20-30 species of megachilids belonging to the genera Osmia and Megachile
under field conditions and the paucity of data which are available in both
the published record and in museums a detailed listing of the species known
to occur in the Upper Peninsula is being compiled. This will include
county records or more detailed specific locality records when available,
plant associations, and periods of flight activity of both males and females.
When the labeling, mounting, and identification of megachilids collected
this season are completed the species complex for each of the potential
nest sites or hutch placement areas will be ascertained. The identification
of approximately 2000 additional Michigan specimens in the Michigan State
University Entomology Museum are currently underway and will be used to
augment our knowledge on the distribution of the group. These data will
provide a basis for a field key useful for project personnel.

3. Plants Used by Megachilids

Plants are used in three manners by megachilid bees: as a pollen
source for provisions for the larval stage, as a source for construction
materials for nest building (resins, portions of leaves or petals, and
plant down), and nectar may be used as a source of sugars as sustenance
for the adult or in some cases apparently mixed with the pollen. Generally,
the same species of plant is not used for all three endeavors. Thus, a

complexity of plant species are involved. Only by careful field observa-
tions at the height of nesting activity will the investigator be able
to ascertain these differences. The problem is further enhanced by certain
long-lived species of megachilids who outlive the normal flowering period
of a particular plant or by a polylectic species of bee (a bee which
gathers provisions from a variety of plant species -- vs. an oligolectic
species which gathers provisions from a single plant species or group of
closely related species). In either case, there may be a shift from one
plant species to another as the season progresses. These detailed differences
in plant usage are virtually unknown for the numerous species of megachilids.
However, a list of plant genera used by megachilids for nectar or pollen
sources has been compiled and will be used to attain a list of plant species
in the investigative area potentially useful to this group of bees. This
will be of tremendous value in selection of nesting sites and later bio-
logical studies on the group.

4. Previous Literature on Megachilid Biology

Prior literature which gives the detailed information pertaining to
flight time for collecting pollen or other materials, field activity of the
bee, numbers of trips to construct a cell and provision, egg-laying habits,
etc. is virtually non-existent. This is particularly true when these kinds
of data are correlated with the various environmental factors (ambient
monitoring) which impinge upon the life of the bee.

Some information is available on ratio of sexes reared from a nest,
cell length, numbers of cells per nest and other similar measurable factors
either before or after emergence. A review of such literature is currently
underway and such data as are applicable or comparable with those to be
acquired in this study will be entered into the computer for later direct
comparison.

5. Set-Up of Computer Programs

Computer programs are currently being written for entry of various
field data on the biology of the megachilid bees including ambient monit-
oring data. Programs are being written to make correlations between any
single/or multiple event in bee biology and any single/or multiple event
in climatic changes. Concurrently, various forms for field collection of
data are being reviewed, revised, or updated to make the transition from
raw field data to a computer bit a simpler task and less likely for error.
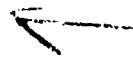
Conclusions

The inability to pursue field activity and collection of data pertaining
to the biology of megachilid bees during the season of 1982 was initially
looked upon with disappointment. However, in looking back upon the situation
it has enabled us to better prepare ourselves for the coming season. We
know that the ecology of the area varies tremendously from one section of
land to another which has brought about a more critical awareness in the
preselection of the 14 experimental sites.

It has enabled the project personnel through collecting in the area to
pin-point the species of Megachile to sections of land in the study area.
Our knowledge of species of the area and their flight ranges will be aug-
mented by the identification of museum specimens and other records. Poten-
tially important plant species will be known. Pertinent biological in-
formation will be summarized from literature sources. Computer programs will
be written and data from other sources will be used to check the programs
and work out any inadequacies in the program before actual field use.
Hutches will be present and preassembled into field units and nest blocks

drilled, assembled, and ready for field distribution. A training program
on the identification of megachilids of the area, the use of various
forms for field observations and collection of data, and a synopsis of the
previous literature on the megachilids of the study area will be conducted
in late spring for all personnel associated with the summer program for
1983.

In short, the time lag in the 1982 season has enabled the Principal
Investigator to know the area, the groups of bees found in the area, the
plant associations of the area, and potential areas for placement of hutches.

Michigan State University
East Lansing, Michigan 48824

Subcontract Number: E06516-82-R-20015

ELF Communications System Ecological Monitoring Program.
SMALL VERTEBRATES: The Michigan Study Site
Tasks 5.6, Small Mammals, and 5.12A, Nesting Birds

Base Period, 1982
First Annual Report

Principal Investigator: _____
Dr. Donald L. Beaver

Co-investigators: _____
Dr. James H. Asher

_____
Dr. Richard W. Hill

_____
Dr. Robert J. Robbins

Releasing Authority: _____
Howard Grider
Contract and Grant
Administration

MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS-1963-A

TABLE OF CONTENTS

GLOSSARY AND ACRONYMS

Antenna system –   Refers to the Extremely Low Frequency (ELF)
                    antenna of the communication system under
                    study in this project.

AOV –   Analysis of Variance. See data-base manage-
                    ment section.

CRPID –   'C'ontinuous 'R'ecording 'P'assive 'I'dent-
                    ification 'D'evice. A system designed for
                    identifying individual animals at the nest
                    box. An animal is "tagged" with a small
                    tuned coil that resonates a specific freq-
                    uency when exposed to an external radio-
                    wave source. No batteries or other outside
                    power source is needed, thus the term
                    passive.

Data logger –   An instrument designed to encode data taken
                    in the field and pass it directly into a
                    computer system. Also referred to as the
                    OS-3 after the manufacturers model number.

Hard disk –   A mass storage device that interfaces with
                    a computer. The usual notation for this
                    device is in terms of the millions of bytes
                    of data that can be stored, i.e. 20 mB or
                    Mega-bytes.

Interscapular –   Referring to a location on the back of a bird or mammal between the scapula or shoulder blades.

Live-trapping –   A technique of censusing small mammals where animals are caught using baited traps which hold them unharmed until they can be tagged and released.

MHz –   An abbreviation for mega-hertz, a unit of measure of radio-wave frequency in millions of cycles per second.

Potted –   Refers to the process of making the tuned coil used in the CRPID unit impervious to body fluids of the animal in which it is implanted. Epoxy and beeswax are typical potting agents.

Resonance –   The response of the tuned coil to radio waves produced by a remote source transmitter. It is this property of the coil that allows an animal to be identified by the CRPID system.

ABSTRACT

Progress on the project to study the effects of the ELF
Communication System on small mammals and nesting birds is
detailed for the base period, 1982. Initial population surveys
were conducted which showed that the main study species, the
Black-capped Chickadee (Parus atricapillus) and the deer-
mouse, (Peromyscus gracilis) were present and abundant on
the pilot plot and several other plots which are potential
sites for establishing permanent plots once the route of the
ELF antenna is known. The pilot plot will serve as a testing
site for studies of parental care, nestling growth and
maturation, fecundity, homing, activity patterns, embryological
development and metabolic physiology. Nesting boxes were placed
on the pilot plot in September and October, 1982, to insure
that animals would be available for these studies in the
spring, 1983. A data-base management system was developed to
coordinate the collection and analysis of data for the planned
studies.

SUMMARY

Progress on the study of the effects of the Extremely Low Frequency (ELF) Communications System on small mammals and nesting birds in the Upper Peninsula of Michigan during the summer and fall of 1982 is described in the following report. The design of the study is multifaceted, looking at a range of attributes of individuals and populations which may be effected by exposure to the radio-waves generated by the communication system. The research scope and plan is detailed in Appendix C.

We have chosen to study the Black-capped Chickadee and the deermouse because both are small bodied, numerous, permanent and breeding residents of the forests in which the communications system will be placed. We have chosen to study population size (in this element of the study we include all species present in the forest, in addition to our main study species), number of offspring born or hatched, parental care of young, growth and maturation of young, development of embryos (for the chickadee only), homing ability after displacement from the nesting site, normal activity patterns, and metabolic physiology (in this case, the ability of the animal to withstand low temperatures).

The study of population size is to be done using standard censusing techniques. For the birds, this involves regularly walking transect lines in prime forest habitat and noting the location of males defending territories in the spring. For small mammals, live-trapping is done on a series of consecutive days with captured animals being marked and released where they

were caught. The number of animals can be determined by constructing a ratio of the number of animals previously caught (the tagged ones) to those not tagged in subsequent sampling. In the 1982 work, we have begun the process of setting up sampling plots and have done preliminary censusing of birds and small mammals (late fall only). These samples indicate that at least 20 species of birds four species of small mammals reside on the plots. Both of the chickadee and deermouse are numerous there.

Other aspects of the study are in the developmental phase. For example, we will monitor the behavior at the nest of chickadees and deermice with a device that can identify each parent, whether it is going into or out of the nest-box, and at what time of the day or night. Additionally, temperature of the eggs, interior of the nest-box and the adjacent out-side will be recorded. This device will allow us to look at more breeding pairs than would otherwise be possible. We will study the homing ability of the test species after they have been taken from the nest and released some distance away. We will examine the 24 hour activity pattern of deermice by placing small radio-transmitters on them. We will collect eggs from the nest of selected chickadees and examine the developing embryos for the presence of abnormalities. We will examine the ability of the test species to produce bodily heat when exposed to low temperatures. All of these aspects of the study will commence in 1983. During the present year, we have made progress toward setting up the equipment to do these studies and we anticipate being fully operational in the spring, 1983.

ACCOMPLISHMENTS IN STUDY ELEMENTS

POPULATION SURVEY - The purpose of this element of the study was to obtain a census of small birds and mammals in test plots near the antenna and control plots distant from the antenna. Our plan for the base period was to locate potential plot sites that could be established in the spring when the final location of the antenna is known. In the interim, we have established a pilot plot on which we will do preliminary censuses. This pilot plot will also serve to supply nesting Black-capped Chickadees (Parus atricapillus) and deermice (Peromyscus maniculatus, hereafter referred to as just Peromyscus) for validation of several aspects of the study to be detailed below.

During the period of 9 September to 25 October, population surveys of birds and small mammals were conducted by Dr. David N. Ewert and Kevin Murphy, with the assistance of Drs. Donald L. Beaver and Robert J. Robbins. The survey was conducted in conjunction with a search for study plots. Three plots in maple-basswood forest (see Appendix A for major tree species composition) were located that were large enough to meet out requirements for plot size. All of these plots contained fall flocks of Black-capped Chickadees and two of the three had good populations of Peromyscus, as determined by live-trapping.

Other species of birds and small mammals were present on these plots as well. For birds, 20 species were observed; the most abundant were: Downy Woodpecker, Hairy Woodpecker, White-breasted Nuthatch, Red-breasted Nuthatch, Blue Jay, Common Raven, Golden-crowned Kinglet, Brown Creeper and Dark-eyed Junco. For mammals: red squirrel, cottontail rabbit, and snow-shoe hare were observed (see Appendix B for scientific names of these species). We have, therefore, met our plan to find and establish pilot plots for the population survey element. Negotiations for final location of plots is presently underway. The exact location of the antenna route is still to be determined, and this will ultimately determine the location of test and control plots. We have, however, consulted with officials of the Michigan Department of Natural Resources, Division of Forestry, to determine the planned use of the forest in the probable path of the antenna and in the location of our pro-posed plot sites, and with officials in the Division of Wild-life concerning the presence of endangered or threatened species of animals. These negotiations will continue into the option period.

PARENTAL AND NESTLING BEHAVIOR, GROWTH, MATURATION AND FECUNDITY - In these elements of the study, we plan to examine the young produced by Black-capped Chickadees and Peromyscus.

We combine the report on the progress in these elements here because we are still in the phase of locating plots and acquiring and developing the equipment necessary to conduct the study.

We have located the pilot plot on which the work will commence in the spring, 1983, and nesting boxes for birds and small mammals have been placed on it. The plot is in sugar maple and basswood forest (described above and in Appendix A). It is about 500 by 200 meters, and contains 44 chickadee and 44 Peromyscus nesting boxes. Preliminary surveys indicate the presence in late September of three resident flocks of Black-capped Chickadees that may use the boxes for breeding in the spring. Initial live-trapping of Peromyscus produced lactating females on the plot and a nearly 50% capture rate per trap-night. We are confident, therefore, that we will have study animals to work with in the spring on this plot.

The equipment for this aspect of the study is in the process of being constructed and tested. We have received the data loggers and initial testing is under way. The unit that will allow us to follow the movement of marked individuals into and out from the nest, (CRPID unit, developed by Dr. Tracy Allen, University of California, Berkeley, and re-designed by him specifically for use in this project), is in the prototype stage. This unit consists of a transmitter that emits a broad frequency pulse in the MHz range, a tuned coil implanted in the test animal that resonates a narrow frequency band in response

to the transmitted frequency, and a receiver which picks up the frequency resonated by the tuned coil (Figure 1. See also Bernd Heinrich, Bumblebee Economics, Harvard University Press, 1979, pp. 110-114). The implanted animal is therefore identified by the frequency resonation of its tuned coil whenever it enters or leaves the nest-box. These data are encoded in the data logger with a time of the event attached.

Complete activity records of marked animals at the nest-box is, therefore, possible. Initial testing of the prototype CRPID in October was very successful. Implanted laboratory mice and Zebra Finches were shown to be detected unfailingly by the system, even when two animals entered the nest-box simultaneously, as mice frequently do. Contributing to this aspect of the study in addition to Dr. Allen has been Dr. Robert J. Robbins, Dr. Richard W. Hill, Dr. Donald L. Beaver, Dr. David N. Ewert, and Kevin Murphy.

Dr. Hill was the principal contributor to the implanting technique, and since the technique is at present in a testing stage, we here discuss it in more detail. The coil was first potted in epoxy and beeswax before implanting. Two adult and two nestling Peromyscus, and two Zebra Finches (being used as test animals in lieu of chickadees because of their similar size and tameness) were surgically implanted with coils. In

Figure 1. Diagram of the tag system developed by Dr. Tracy
Allen, University of California, Berkeley. A. The loops shown
are the transmitting antenna, attached to the scanning
transmitter, and the recieving antenna (at right angle to
transmitting antenna) which sends signals to the UHF dectector.
Depicted in B is a bumble bee, the animal for which the system
was originally developed, with a tag mounted on its back. The
tag is a tuned coil that resonates at a specific frequency when
it is hit by the broad spectrum frequency band produced by the
transmitter. The frequencies are very high, in the ghz range.
Depicted in C is the linear output of the transmitter and in D
the response of the tuned coil. The information received from
the tuned coil is analysed by an amplifier and detector unit, a
peak position detector unit and finally a scan generator and
data latch unit before the data are output to a recorder.
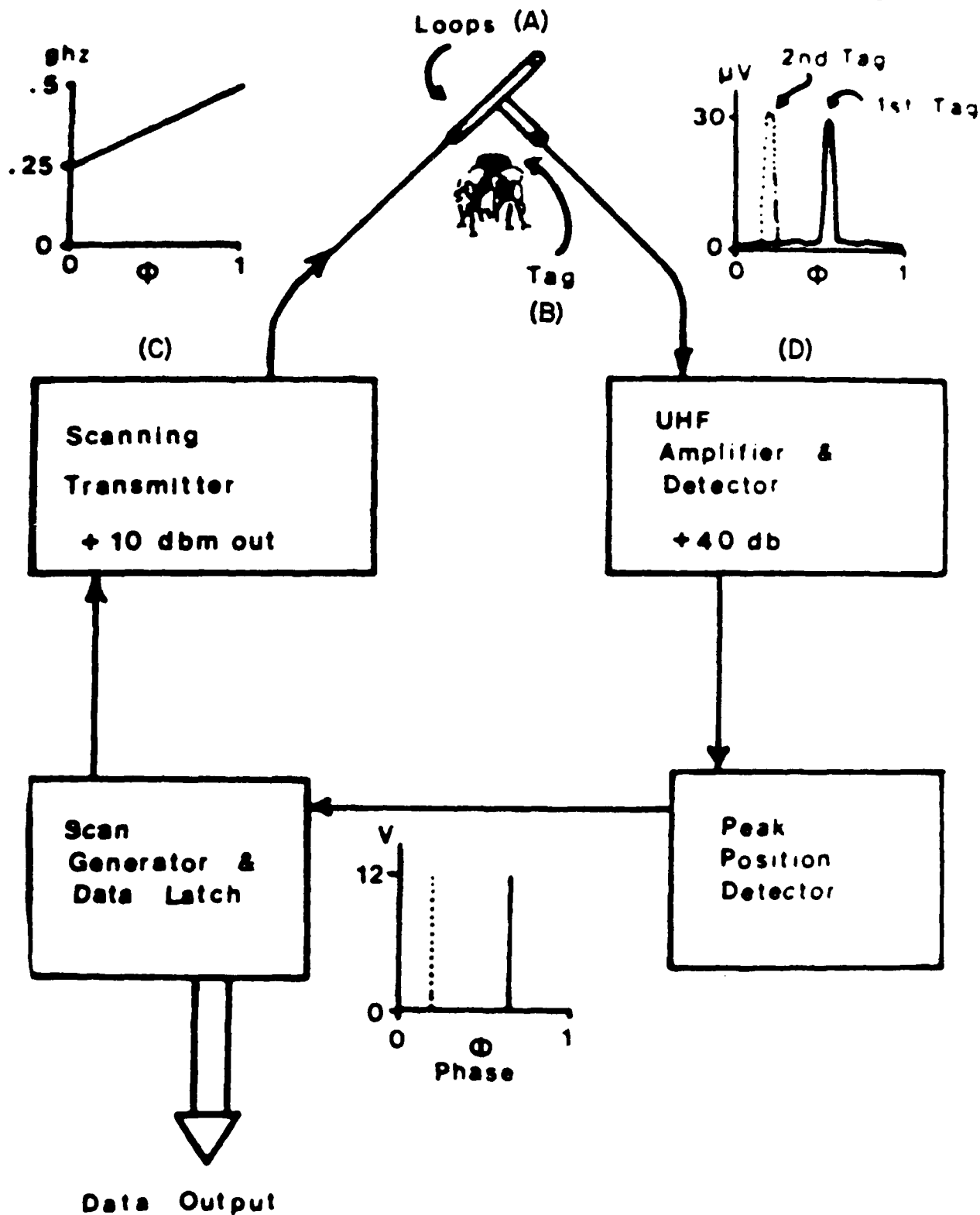
Tag System Diagram

carrying out these implantations, implant sites have been evaluated; for both the birds and mammals, an interscapular site seems likely to prove best. Surgical techniques have also been perfected. All animals have survived the surgery in good immediate health. At this writing -- three weeks after the surgery -- all except one remain in good health; one bird died, but the circumstances suggest that the cause of death may well not have been related to the surgery or the implanted coil.

Besides being used to evaluate and perfect the surgical implantation procedure, the implanted animals are also being used in testing of the CRPID system. The animals have already served as test subjects in evaluating the efficacy of various antenna configurations. By maintaining the animals over the months ahead, we will also evaluate whether the protracted exposure of the resonant coils to body fluids and body movements will cause drift in their resonant frequencies. Ultimately, we will reenter these animals surgically to verify that the coils have not precipitated undesirable tissue reactions at the implantation sites.

A continuing program of such testing, using additional animals, is planned.

Other aspects of the study of parental care and nestling growth and maturation will not be undertaken until spring, 1933, when we expect to have breeding individuals in the nest boxes.

HOMING AND ACTIVITY PATTERNS- This element is planned to begin in the spring, 1983. Homing will be examined by displacing tagged chickadees and Peromyscus individuals from the nest and noting the elapsed time to return (or if they return). The return of the tagged individual will be monitored by the CRPID unit. Activity of Peromyscus will be additionally monitored by placing small radiotransmitters on selected individuals. A receiver will be used to locate these individuals on an hourly schedule over a number of days so that their use of space can be detailed.

• The study of homing behavior in the Black-capped Chickadee and the deermouse is to be accomplished by use of the CRPID device and was therefore delayed until these units can be developed and tested. We plan initial field testing in the spring, 1983. The telemetry equipment to be used for detailed study of activity patterns of Peromyscus arrived in late September. Initial testing began in October with the planned trip to the Clam Lake, Wisconsin, site. At this site, the mouse-sized transmitters will be field tested to see if there are any synergestic effects with the ELF transmission. At the writing of this report, the results of these tests are not yet in. Contributing to this aspect of the study are Dr. Robert J. Robbins and Kevin Murphy.

DEVELOPMENTAL BIOLOGY - The thrust of this aspect of the study was to examine the normal developmental patterns of Black-capped Chickadees, about which little is known, and compare the patterns discerned on the test and control plots. The study involved obtaining known aged embryos from nests of chickadees in the field and preparing them for gross and detailed examination in the laboratory. The equipment needed to determine the age of the embryos is a simple temperature recorder that will indicate the start of incubation, and thereby the start of development.

The main activity scheduled for this aspect of the study during the base period was the acquisition and testing of equipment. At this writing, only the thermistor probes have arrived. The Rustrak recorders are delayed because the internal drive motors had to be altered to run on 6 volts DC, a requirement for field operation. We are confident that they will soon arrive and testing can be accomplished before their use is required in spring, 1983. Contributing to this aspect of the study are Dr. James H. Asher and Dr. Donald L. Beaver.

METABOLIC PHYSIOLOGY - In July and August, considerable effort was directed to apprasing the exact design properties of each piece of equipment planned for use in metabolic studies. The overall goal of these investigations was to assure that the equipment, once ordered, would function exactly as required. Two subsidiary goals were (1) to verify that various pieces of

equipment would interface properly and (2) to assure that equipment for gas handling and analysis would function correctly with the unusual gas mixture to be used in the metabolic research (21% oxygen, 79% helium). Technical bulletins on equipment were read, and manufacturers' technical presonnel were consulted extensively, in pursuit of these goals.

Based on the above investigations, a set of equipment was ordered with the conviction that the metabolic apparatus, once assembled, would provide a high degree of accuracy, convenience, reliability, and durability in the applications planned. A schematic representation of the equipment and experimental design for the study of metabolic physiology is shown in Figure 2.

Since mid-September, equipment has been arriving. Each item has been examined carefully on arrival. All key items are expected to have arrived by the middle or end of November, and the entire apparatus will them be assembled and thoroughly tested in East Lansing prior to being transported to the research site in the Upper Peninsula.

DATA-BASE MANAGEMENT - In all of our approach to data-base management, we have been guided by two principal goals. First, we have assumed that the project may be a long-running one and that consequently all data-base management and manipulation

Figure 2. The metabolic apparatus for measuring peak thermal
output. Depicted in A is the system for delivering a Helium-
Oxygen atmosphere to the subject animal. Depicted in B is the
chamber and thermal water bath that will house the experimental
animal and control the temperature during the test. Depicted in
C is the valve system that will allow selecting the output gas
of respiration from up to three subjects being tested at once,
and in D the analysis of the gas in the oxygen analyser unit
and the output of the data to the recorder.
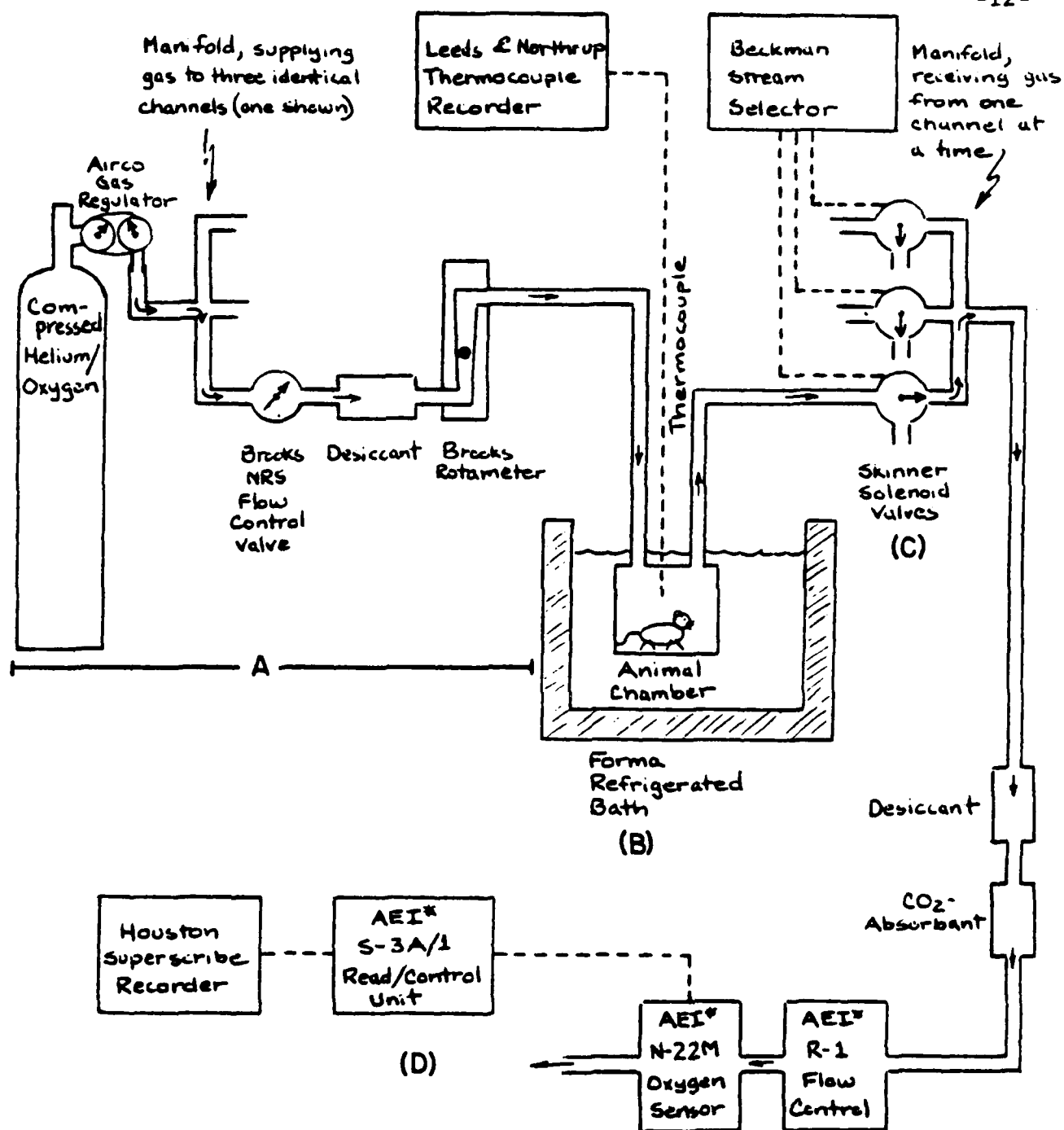
METABOLIC APPARATUS

techniques should be designed for personnel independence. That is, we are striving to develop sufficiently standardized and automated methods for data management so that personnel changes will not lead to changes in data storage protocols. Second, we have assumed that new ideas for specific analyses and comparisons of the data will be generated throughout the duration of the project, and possibly beyond. Therefore, we are designing our data storage methods so that all data will be indexed and cross referenced so that meaningful subsets of data may be easily extracted from the overall data set.

Equipment:

Although we initially proposed to use an Altos 8600 series computer for our data-base management and analysis, we have decided to switch to the IMS International 8000-S series computers. We feel that the 8000-S will better serve our needs for flexibility, as it is an S-100 bus system and thus can be configured differently as our needs change.

Initially, our units will have 20 mB hard disk drives for mass data storage and twin 8-inch floppy disk drives for back-up copies of data. As our data base accumulates we will be able to add more hard disk storage and a 17 mB tape cassette system for large-scale data back up storage.

Data Management:

We have attempted to use an integrated approach to all three aspects of data-base management (data acquisition, data storage, and data analysis), so that we may better attain our goal of flexibility and long-term usefulness of the data base.

Acquisition:

In our experimental work we will acquire data both automatically and manually that will ultimately have to be entered into our data-base management system. To insure that no idiosyncracies are introduced into data storage formats, all of our data entry will be accomplished through a program interface. Data from our OS-3 data loggers will be dumped to a running program for initial analysis and formatting, before being stored on disk. All manually entered data will also be accomplished through an interactive program which will perform some error checking and will make sure that all data entries are properly formatted before being stored on disk. All data files, whether on hard or soft disk, will contain header records that (a) identify the data, (b) give the formatting of the data records in the file, and (c) explain the meaning of the coding in the data records.

Storage:

To facilitate cross indexing, data sorts, data subset generation, etc., each data record will contain sufficient indexing and coding so that the dependent measures in the record may be assigned to a variety of hierarchically arranged analysis categories, depending upon the goal of the particular analysis being carried out. Although the redundant storage of "header" information in each data record will be somewhat expensive in terms of storage space, we believe it is justified in that it will facilitate data sorts and, more importantly, it will ensure the integrity of these "header" codes in the event of a partial data loss.

Analysis:

We plan to use analysis of variance as our primary statistical tool in examining metric data. To this end, we have developed a general-purpose analysis of variance routine that is capable of handling unlimited numbers of data points in a design containing up to 100 separate treatments. The only requirement is that the data be presented as a series of records, with one field of the record containing a value indicating the treatment group and another field containing the data value to be analyzed. The routine automatically provides an overall F test for differences among the treatment groups and it also automatically provides summary measures (mean., standard deviation, and standard error) and summary infor-

mation (N, sum of x, and sum of x-squared) for all groups. In addition, the routine allows the generation of an all-ways orthogonal analysis of individual contrasts between pairs or sets of treatment groups, using user-specified contrast vectors. It also permits the analysis of additional, nonorthogonal contrasts, if desired.

The AOV program is written to run interactively, using user-specified parameters to set up the design. An additional program is being written that will also run interactively and will allow the user to attach a major data file and to extract from that file the appropriate data records for the analysis at hand. In addition, this set-up program will allow the user to specify the appropriate independent-variable values for each treatment group and then it will examine the data file, identify the data records with acceptable independent-variable values and will assign the appropriate temporary (i.e., for this data analysis) treatment-group number to each data record. Thus, the data-subset file generated by this program can be used directly as the data file for the analysis of variance program. After creating the data file, the set-up program will interrogate the user and will create a file containing the desired set of contrast vectors. Standard sets of all-ways orthogonal contrast vectors will be available, but user-specified sets may also be used. Contrast-vector sets will be checked for all-ways pairwise orthogonality.

Specific accomplishments to date:

As per our original proposal, we have been preparing for our first field season to begin next spring. Thus, we do not currently have any actual data requiring analysis and we will not be carrying out analysis of field-generated data until the field season begins.

Our computer hardware is currently on order, although it has not yet (as of 23 October) arrived. The analysis of variance routine has been written in Fortran IV on the University's mainframe Cyber 750 and will be modified for the Fortran compiler on the IMS 8000-S. The Observational Systems OS-3 data loggers have arrived and have been tested and are currently being tested for data delivery to a host computer. Meetings have been held with field staff and project directors to begin developing an understanding of the data formats that will be required for all of the different experimental work. A design for the set-up program has been drafted. Accounting software for maintaining project records has been developed both for the University's mainframe machine and for microcomputers.

With the arrival of the IMS 8000-S equipment, programming will begin in earnest, with program details being modified for optimal performance on the IMS system. At this time, we do not anticipate any delays or difficulties in being fully ready to receive and analyse data with this system in the field season beginning in April, 1983. Dr. Robert J. Robbins and Dr. Donald L. Beaver have dealt with the development of the data-base

management system primarily, but valuable contributions have been made by all the investigators and research personnel.

CONCLUSIONS

In the base period of the contract, we have established a pilot plot on which we placed nesting-boxes for Black-capped Chickadees and _Peromyscus_. This plot will be used for testing the CRPID device as well as obtaining preliminary data on all elements proposed for study. We have located other potential plot sites for the eventual establishment of plots for the study of parental care, and growth and maturation of young. We have acquired equipment for most aspects of the study and anticipate implementation to the full level of our revised proposal in the spring, 1983.

APPENDIX A -19-

Tree abundance on the pilot plot. The data are based on sampling the nearest tree at 50 m intervals along four parallel transects 100 m apart.

Tree species	Number	% of total
Sugar maple (Acer saccharum)	26	65
Basswood (Tilia americana)	7	17.5
Balsam fir (Abies balsamea)	3	7.5
Black cherry (Prunus serotina)	2	5
American elm (Ulmus americana)	1	2.5
Bigtooth aspen (Populus grandidentata)	1	2.5

APPENDIX B

Scientific names of the most common birds seen on the pilot plot in September and October, 1982.

Common name	Scientific name
Downy Woodpecker	Picoides pubescens
Hairy Woodpecker	Picoides villosus
Blue Jay	Cyanocitta cristata
Northern Raven	Corvus corax
White-breasted Nuthatch	Sitta carolinensis
Red-breasted Nuthatch	Sitta canadensis
Brown Creeper	Certhia familiaris
Golden-crowned Kinglet	Regulus satrapa
Northern Junco	Junco hyemalis

APPENDIX C.   Small Vertebrates: The Michigan Study Site

SMALL VERTEBRATES: The Michigan Study Site

RESPONSE to RFP: IITRI E06516-82-R-00015

TASK GROUPS 5.6 and 5.12: Small Mammals and Nesting Birds

TECHNICAL VOLUME

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Preface to the Technical Volume

The experiments proposed in this volume are designed to answer questions related to two tasks as defined in the Solicitation No. IITRI-E06516-82-R-00015, viz. Task 5.6, Small Mammal Biometric Survey, and the nesting-birds portion of Task 5.12, Nesting Birds and Migrating Birds in Flight.

In this Technical Volume we outline a coordinated and integrated approach to the investigations on both small mammals and nesting birds, as we believe that such an approach is scientifically superior to a study of each task separately. Additionally, we show in the Budget Volume that such an approach is also cost effective.

Small mammals and nesting birds constitute a single, logical category of small, homeothermic vertebrates and as such they are amenable to a unified program of investigation. Many aspects of their biology (such as, parental behavior, fecundity and reproductive success, growth and development of progeny, homing ability) can be investigated with similar techniques, thereby allowing the acquisition of a similar and comparable data base with which to investigate the effects of ELF radiation upon a natural ecosystem. The use of consistent investigational techniques across birds and mammals not only insures comparability of results, but it also permits considerable savings in both personnel and equipment costs, with no loss in scientific accomplishment.

Because the unified, coherent nature of our proposed research program can best be judged through an examination of the entire proposed research project, we have provided an integrated description of our proposed work in the Technical Volume. For the convenience of reviewers, we have indicated in the Table of Contents the specific sections that apply to mammals and those that apply to birds. In the Budget Volume we have provided a budget for the combined birds and mammals work and in addition we have provided separate budgets for the two tasks as defined in the Solicitation.

TECHNICAL VOLUME, PART 1

SCIENTIFIC APPROACH

Section 1.   Introduction

The purpose of this proposed research is to determine if the electromagnetic fields and gradients produced by an ELF Communication System affect small mammals or nesting birds living in the forest environments in or near the system, pursuant to IITRI Solicitation No. E06516-82-R-00015 as amended. The studies proposed are planned to extend for a period of at least 5 years so as to encompass years both preceding and following the construction and initiation of operation of the Communication System. The studies are planned for the Michigame, Escanaba, and Ford River State Forests in the Upper Peninsula of Michigan.

Heretofore, effects of ELF electric and magnetic fields on many attributes of birds and mammals have been studied extensively in the laboratory (see reviews by Persinger et al., 1973; Kaufman and Michaelson, 1974; Persinger, 1974, 1976; Marino and Becker, 1977; National Academy of Sciences, 1977; Sheppard and Eisenbud, 1977; Schiff, 1978; Phillips et al., 1979; Tenforde, 1979; Grisset, 1980). However, relatively few studies have been carried out on the effects of ELF electric and magnetic fields on birds and mammals living in their natural habitats. Further, studies carried out heretofore on natural populations have to a large extent been limited to assessment of animal abundance (e.g., Seale et al., 1976; Anderson et al., 1977); such assessments are uniquely useful

in certain respects (see later), but as Seale et al. (1976) point out, mere measures of abundance are unlikely to reveal any but "nonsubtle" effects and some studies of abundance have not been properly controlled. With a few notable exceptions (e.g., Southern, 1975; Larkin and Sutherland, 1977), experimental or well-controlled descriptive studies have not been carried out on particular behavioral, developmental, or physiological attributes of birds and mammals exposed to ELF fields in their natural environments.

Studies of potential ELF effects in natural environments are necessary to supplement laboratory studies because of limitations intrinsic to the laboratory studies. For example, certain types of critical behavior, such as food-gathering or homing, cannot be simulated well in the confines of a laboratory enclosure. Many of the species of animals living near the ELF Communication System either do not prosper at all or fail to reproduce in the laboratory, meaning that some or all of their significant attributes cannot be studied in a laboratory setting. Indeed, it is noteworthy that the species used heretofore in laboratory studies (e.g., chickens, laboratory rats, laboratory mice, and rhesus monkeys) generally do not occur in the vicinity of the proposed ELF Communication System, and few of the species that do occur in that vicinity have ever been studied in the laboratory. In further defense of the need for studies in natural environments, we would note that many processes are subject to very different constraints in laboratory and natural settings. For example, rates of postnatal growth and development of altricial young are strongly dependent on

the effectiveness with which parents provide both food and warmth. In a laboratory cage where food is freely available, parents have no difficulty meeting the nutritional requirements of nestlings; further, as a consequence of the ease of obtaining food, they can spend less time foraging and more time in the nest than in natural environments, thereby warming the young more extensively (Hill, 1972b). To the degree that ELF fields might interfere with parental foraging, they might indirectly affect the growth and development of nestlings by impairing provision of nutrition or warmth. Such effects would seem much more likely to become evident in natural environments than laboratory ones, given that parents intrinsically have a more difficult time providing adequate nutrition and warmth in the natural settings. Accordingly, rates of growth and development of nestlings exposed to ELF fields need to be assessed in natural environments even though they have already been studied in the laboratory (e.g., Durfee et al., 1976; Giarola et al., 1972; Knickerbocker et al., 1976; Krueger et al., 1972; Krueger and Reed, 1975; Marino et al., 1976a, 1976b; Mathewson et al., 1979; Noval et al., 1977; Ossenkopp and Shapiro, 1972; Ossenkopp et al., 1972; Persinger et al., 1973; Smith et al., 1977). The same can be said of many other parameters such as behavior (e.g., Altman and Lang, 1974; Baum et al., 1975; deLorge, 1973; Gavalas et al., 1970; Gavalas-Medici and Day-Magdaleno, 1976; Grissett, 1971; Grissett and deLorge, 1971; Marr et al., 1973; Ossenkopp, 1972; Persinger, 1969; Persinger and Pear, 1972; Persinger et al., 1972, 1973).

The proposed studies will be carried out almost entirely on animals living in the forest environments surrounding the ELF

Communication System in Michigan. Studies of laboratory-housed animals will be limited to those needed for perfection of techniques that will ultimately be applied to free-living animals. Besides monitoring animal abundance near the site of the ELF Communication System, we plan detailed studies of many behavioral, developmental, and physiological parameters that we deem likely to be especially sensitive to disturbance. All species studied will be native to the environs of the ELF Communication System.

We propose to study both nesting birds and small mammals. Accordingly, our proposal encompasses one entire work task (5.6 - Small Mammal Biometric Survey) and part of another (5.12 - Nesting Birds and Migrating Birds in Flight). We have combined these two work elements because of the unique talents of the senior scientists involved and because we believe both should be approached with very similar techniques. We project considerable economic savings by using the same equipment in studies of both groups of animals. Further, by gathering similar types of data on both birds and mammals, we expect to be in a superior position to evaluate impacts of ELF fields on any one species from a comparative viewpoint.

Section 2.   Rationale For Proposed Studies

Dozens of species of small birds and mammals are likely to be resident near the ELF Communication System, and the operation of the Communication System could in principle affect any of them in any of a number of ways. Even with virtually unlimited resources, it would be impossible to monitor individually all ecologically important aspects of all species for effects of the Communication System.

Should the Communication System have negative effects, many of them could be expected to reduce the population density of the species involved. For example, population density would tend to be reduced by such diverse impacts as a decrease in fertility, an increase in developmental abnormalities, a decrease in parental attentiveness to the young, or an impairment of the ability of animals to detect and avoid predators.

In view of the susceptibility of population density to manifold effects, we plan to survey the densities of most species on a regular basis by live-trapping and visual observations. There is no better way for us to be in a position to detect diverse potential effects in the many taxonomically disparate species resident near the ELF Communication System.

While measures of population density are uniquely useful for the above reasons, they have a significant limitation as well, namely that effects on density are relatively difficult to detect in practice. The population densities of birds and mammals are inherently variable (e.g., Andrewartha and Birch, 1954; Kluijver,

1951; Lack, 1954, 1966; Perrins, 1965; Pianka, 1978; Ralph and Scott, 1981). When comparing a parameter at two locations, statistical principles dictate that, to the degree the parameter is variable, the number of independent measurements taken at each location must be increased to achieve a particular likelihood of detecting differences that exist. Because population density is highly variable, the number of independent measurements of density at sites near the ELF Communication System and at control sites should be large to have a high probability of detecting modest differences between the two types of sites. Yet large numbers of independent measurements of density are difficult to obtain because density is a property of populations, and a functionally coherent population of birds or mammals may be highly dispersed, occupying a sizable area of field or forest. For example, intensive sampling of a 15-hectare plot will yield one estimate of the density of flying squirrels; hence, the total area that would need to be sampled to obtain truly large numbers of independent estimates of density would be prohibitive.

In short, study of density has significant merits and limitations. The most important limitation is that the number of independent measures that can be obtained in practice is sufficiently small relative to the intrinsic variability of the parameter that only sizable effects of the ELF Communication System on density will be statistically detectable. On the other hand, many types of possible primary ELF System effects might secondarily affect bird and mammal density. Thus, by monitoring the densities of most species of small birds and mammals, we will substantially

assure that if the System has a sizable deleterious effect on any one attribute of any one of the diverse species present, we will be in a position to detect the effect. This is important in view of the impossibility of monitoring directly all attributes of all species.

Even though effects of ELF electric and magnetic fields have been studied extensively in the laboratory (see reviews by Persinger et al., 1973; Kaufman and Michaelson, 1974; Persinger, 1974, 1976; Marino and Becker, 1977; National Academy of Sciences, 1977; Sheppard and Eisenbud, 1977; Schiff, 1978; Phillips et al., 1979; Tenforde, 1979; Grisset , 1980), many animal attributes and many taxonomic groups resident in northern Michigan have never been examined -- thus the need to screen for sizable effects as planned by monitoring population densities. Nonetheless, laboratory research indicates that if the ELF Communication System has effects on birds or mammals, the effects will be small, and a statistically robust experimental design will be required to detect them. Thus, much of our work will be focused on a detailed study of particular animal attributes with the aim of gathering a sufficiently large set of data to detect small differences that might exist between sites near the Communication System and control sites.

In selecting the particular attributes to be intensively studied, we have been guided by three major considerations:

1. We plan to study parameters that can be measured on
    individuals rather th populations. Large numbers of
    measurements can be readily obtained on attributes of
    individuals, thus facilitating statistical detection of any

effects the ELF Communication System may have.

2. We plan to emphasize ecologically significant parameters that are especially likely to be susceptible to perturbation. Reproduction and development, for example, will receive particular attention because they not only are demographically important but also are more likely to be sensitive to aversive environmental changes than many other animal properties (e.g., Goodposture, 1955; Koskimes, 1950; Kluijver, 1951; Krebs, 1970; Lack, 1954, 1966; Nice, 1954; Perrins, 1965; Perry and Rowlands, 1973). Behavior will be studied in depth because it is sometimes readily modified, and yet such modifications can have major repercusions on the lives of individuals and populations (e.g., Cohen et al., 1980; Green, 1979; Morse, 1980; O'Connor, 1978; Slobodkin, 1968).

3. To avoid an overly wide-ranging and dilute study, we plan to concentrate our efforts on four, relatively abundant species of small mammals and nesting birds. We have selected the species carefully with a view to maximizing their ecological and taxonomic diversity, so as to maximize the probability of detecting whatever diverse effects the ELF Communication System may have.

Our experimental design, planned methods of statistical analysis, and specific selections of species to be studied will be discussed in detail at the start of Section 3 (Proposed Studies). Here we shall now discuss in greater depth the particular biological parameters to receive attention.

Section 2.A. Behavioral Studies.

In view of the established sensitivity of certain types of
orientational behavior to alteration by ELF fields (e.g., Graue,
1974; Keeton et al., 1974; Larkin and Sutherland, 1977; Southern,
1969, 1971, 1972a, 1972b, 1973, 1974, 1975, 1976) orientation and
homing in small mammals and nesting birds will be tested to see if
they are affected by the ELF Communication System. Specifically,
the ability of animals to return to their home-range or territory
after displacement will be assessed. Animals are able to find food
(Krebs, 1970; and Royama, 1966) and escape predators (Metzgar,
1967; and Watson, 1964) more effectively in their home-range or
territory than in less familiar areas. Thus, any disturbance of
their ability to return to their home-range or territory after
wandering afar could decrease their probability of survival.

The attentive behavior of parents will be assessed by
monitoring visits to the nest containing eggs or young. Disturbance
of attentive behavior could impair development of eggs or nestlings
inasmuch as the latter are dependent on parents for both food and
warmth (e.g., Balen and Cove, 1972; Hill).

Movements of nestlings to and from the nest will be
monitored. In birds, nestlings typically remain in the nest until
they fledge, and thereafter they do not return to the nest although
they may remain in its vicinity (Bent, 1942, 1946; Lack, 1966).
We will determine the age of fledging. Nestling mammals undertake
temporary trips away from the nest before leaving the nest for good
(Galef, 1971; Alberts and Leimbach, 1980; Vestal, et al., 1980).

We will monitor the frequency and duration of the temporary trips as well as the age of weaning. Knowledge of the fledging or weaning age is important because size and maturity at that age affect the probability that the young animal will survive to reproductive age (Lack, 1966; Perrins, 1965; Murphy, 1978). Measures of the temporary trips made away from the nest by nestling mammals are significant because the young learn to find food and orient during those trips (e.g., Galef, 1971). Impairment of such learning could decrease the probability of survival once weaning has taken place.

The free-ranging behavior of adults will be monitored to assess the frequency and lengths of movements and the spatial distribution of activity within the home-range. Particular attention will be directed to the question of whether space near the Communication System antenna is less used than more-distant space. Such a bias in space utilization would indicate that the electric and/or magnetic fields of the antenna are behaviorally aversive.

The birds to be studied are active during the day, whereas the mammals are active at night. We will monitor, in either case, the times that activity begins and ends and the daily duration of activity.

Section 2.3. Reproduction, growth, and development.

The frequency and type of prenatal developmental abnormalities will be examined in birds (mammals will not be studied in this respect because reproductive females would have to be killed to examine fetuses, and such deaths could have serious, adverse effects on population demographics). Prenatal developmental stages are especially likely to be susceptible to environmental perturbation (Axelsson, 1954; Saxen and Rapola, 1969). There is, at present, no evidence to demonstrate that electric and magnetic fields of the magnitude generated by the ELF Communication System are capable of causing embryonic or fetal developmental defects. Because of the very low energy levels involved, it is unlikely that ELF fields could cause such defects directly. On the other hand, brooding temperatures are extremely important for normal avian development. In particular, eggs must be kept warm by parental incubation in the field; and should incubation behavior be disturbed by the ELF Communication System, developing eggs might experience abnormal reductions in tempera- ture. It is well established that such hypothermia could induce developmental abnormalities (Zwilling, 1956; Hamilton, 1965).

We will monitor fecundity in both birds and mammals by counting both the number of broods or litters produced per year and the number of viable young per brood or litter. Fecundity is an important parameter to study not only because it is demographically significant but also because it influenced by a number of parameters that could, in principle, be affected by the ELF Communication System. Alteration of male or female reproductive

physiology could affect fecundity. Further, any serious disturbances of prenatal development in mammals or birds would likely be reflected in a decrease in fecundity inasmuch as abnormal fetuses frequently fail to be born (i.e., they are resorbed in utero or fail to hatch) or are eaten or discarded by the parent soon after birth. To study fecundity, nest-boxes will be provided, and the reproductive output of adults electing to nest in the boxes will be monitored.

The postnatal growth and development of young reared in nest-boxes will be followed. Any effects that the Communication System might exert on the young themselves could be reflected in altered rates of growth or development. Alternatively, disturbances of parental attentive behavior could be influential because the rates of growth and development of nestlings are dependent on the extent to which parents provide not only food but warmth (Hill, 1972). As a subpart of our study of growth and development, we will give particular attention to the state of maturity of young at the time of weaning or fledging. When young wean or fledge, they must become substantially self-sufficient; thus their maturity can affect their likelihood of survival. There is evidence that young birds that fledge when of relatively low body size are significantly less likely to survive than ones that grow to larger size while still in the nest (Lack, 1966; Murphy, 1978). Postnatal mortality of nestlings will also be monitored.

Section 2.C. Thermoregulation.

In the region of the ELF Communication System, low

temperatures make winter the most physiologically stressful time of year, at least for animals such as chickadees and squirrels that live wholly or predominantly above the snow. We will measure two parameters that will index the ability of such animals to cope with the severity of the winter climate. Deficits in an animal's physiological ability to thermoregulate would be expected to decrease the animal's probability of survival to the next reproductive season.

Birds and mammals keep warm by producing heat metabolically to offset heat losses. The extent to which they can keep their body temperature above air temperature depends on how rapidly they can produce heat. In other words, the lowest air temperature at which they can maintain their usual body temperature is a function of their maximal rate of heat production (Hart, 1957). In view of these principles, we shall measure the maximal rate of heat production of selected birds and mammals during winter. This peak rate of heat production is informative not only because it determines the lowest air temperature at which thermoregulation is possible but also because it provides an index of metabolic endurance. The higher an animal's maximal rate of heat production is, the longer the animal will be able to maintain any particular submaximal rate of heat of production (Astrand and Rodahl, 1977; Wickler, 1980). Endurance is important because low air temperatures demanding high heat production can persist for long periods of time. Beyond its immediate significance for survival in a cold climate, the maximal rate of heat production is a valuable parameter to measure because it reflects deficiencies that might

arise in numerous physiological systems. The ability of the respiratory system to provide oxygen, the ability of the circulatory system to transport both oxygen and nutrients to metabolically active tissues, the ability of storage tissues (e.g., adipose tissue) to mobilize stored nutrients, the enzymatic competence of metabolically active tissues to catabolize nutrients, and many other parameters can affect an animal's peak rate of heat production (Wang, 1978). Thus, the peak rate of heat production itself provides a holistic indication of the performance capabilities of many physiological systems.

Another factor that determines whether an animal can keep itself warm is the availability of metabolic fuel. Both birds and mammals have inactive phases each day, during which they do not collect food and rely, largely or completely, on stored fat. Night is the inactive phase for most birds and for mammals such as squirrels. These animals store fat while feeding during the day and use the fat at night; and in some cases, during cold weather, the fat stores available in the evening may be little greater than those necessary to sustain the animal until dawn (Chaplin, 1974; Blem, 1976). Because available fat stores may be only marginally adequate, even modest reductions in those stores can, in some instances, significantly lower an animal's chances of survival; that is, the size of the fat store accumulated each day can be a sensitive indicator of an animal's ability to cope with winter cold. Accordingly, we will monitor the fat stores of selected birds and mammals. The size of the stores accumulated each day could be altered not only by physiological disturbances but

also by changes in the animal's behavioral ability to acquire food. The acquisition of food can involve search behavior, capture behavior, and the ability to orient to stores of food or other rich food sources.

Section 3.   Proposed Studies

Section 3.A.   Specific questions to be answered. Each question will
be dealt with in detail in Section 4. (Actual Experimental
Studies).

A. Do the electrical and magnetic fields of the ELF
    Communication System alter the population density,
    species composition, or distribution of small birds or
    mammals?

B. Do the electrical and magnetic fields of the ELF
    Communication System alter orientation and homing
    abilities?

C. Do the electrical and magnetic fields of the ELF
    Communication System alter parental behavior?
    Specifically, do they alter the time parent birds
    spend brooding, incubating and feeding their young and
    the time parent mammals spend nursing and warming their
    young?

D. Do the electrical and magnetic fields of the ELF
    Communication System alter the rate of growth and
    maturation of nestlings? Specifically, in birds do they
    alter:

    1. the weight of eggs laid?
    2. the length of the incubation period?
    3. the timing of hatching of the young?
    4. the growth of the nestlings?
    5. the age at eye opening?
    6. the age of feather appearance and growth?
    7. the age of fledging?

Specifically, in mammals do they alter:

9. the growth of the young?

10. the age at eye opening?

11. the age at eruption of lower incisors?

12. the age at opening of the external auditory meatus?

13. the age at first departure from the nest?

14. the duration and frequency of temporary excursions from the nest?

15. the age of weaning?

E. Do the electrical and magnetic fields of the ELF Communication System alter the day/night activity schedule of adults? Specifically, do they alter in birds:

1. the day/night activity pattern at the nest?

and in mammals:

2. the day/night activity pattern at the nest?

3. the day/night activity pattern away from the nest?

F. Do the electrical and magnetic fields of the ELF Communication System alter fecundity? Specifically, do they alter in birds:

1. clutch size?

2. survival of eggs to hatching?

3. survival of nestlings to fledging?

and in mammals:

4. litter size?

5. length of gestation period?

6. age of first gestation?

7. survival of nestlings to weaning?

G. Do the electrical and magnetic

fields of the ELF Communication System alter prenatal

development in resident birds?

Specifically, do they alter:

1. the occurrence of prenatal developmental

abnormalities?

2. the rate of development of embryos?

H. Do the electrical and magnetic fields of the ELF

Communication System alter thermoregulatory

capabilities. Specifically, do they alter in birds and

mammals:

1. the winter heat-productive capacity?

2. the body-fat-content cycles?

Before considering the questions posed above, we first

consider the selection of bird and mammal species for testing the

effects of the electrical and magnetic fields produced by the ELF

Communication System. We then consider the design of the study

plots, and justify their size based on the constraints of sample

size. That section is followed by a detailed treatment of the

statistical design we plan to use. We then present our actual

experimental studies. We shall present a work plan through

October, 1986. However, regardless of when the ELF Communication System is placed into operation, we plan to continue our studies for long enough to encompass at least 2-3 years of full operation of the System.

Section 3.3. Selection of species for intensive study.

In selecting species of both mammals and birds for inten-
sive study, the two criteria of major concern were: (1) the species
must be abundant in the region where the ELF Communication System
will be built, and (2) the species must be known to occupy and rear
young in nest-boxes. The latter criterion is important because
several aspects of our study will of necessity be carried out on
animals living in nest-boxes.

We plan to carry out our intensive studies on two species
of birds and two of mammals. The two species of each type have been
selected to have significantly different life histories. In this
way, we seek to maximize our chances of detecting effects of the ELF
Communication System, if present, even if those effects should turn
out to be specific to particular aspects of life history.

Section 3.3.1. **Birds.** One bird to be studied is the
Black-capped Chickadee (<u>Parus atricapillus</u>), a year round resident
species in the upper peninsula of Michigan (Zimmerman and VanTyne,
1959). It is likely to be the most abundant resident species in the
forests near the ELF Communication System (VanVelzan, 1980).
It is also known to occupy and rear its young in nest-boxes
(Drury, 1958; Lack, 1955). The second species to be studied is the
Tree Swallow (<u>Tachycineta bicolor</u>). This species is a very common
summer breeding species in forest openings and edges (Zimmerman and
VanTyne, 1959). It readily accepts nest-boxes for breeding. A
significant life history difference between the chickadee and
swallow is that the swallow is highly migratory and spends the
winter months at tropical latitudes. Swallows will, therefore, be

exposed to the ELF Communication System only during the summer months whereas the chickadee will potentially have year-long exposure. Additionally, the swallow family (Hirundinidae) is known for its homing abilities when displaced from the nest (Southern, 1959) whereas chickadees are not (Odum, 1941c).

The Black-capped Chickadee is known frequently to attempt two broods per year. The average clutch size is 6 eggs in first broods and fewer in second (Kluijver, 1961). Tree Swallows have one brood per year with an average clutch size of 6 eggs (DeSteven, 1978; Chapman, 1939).

The expected return frequency of birds marked as breeders on the study plots from year to year is 30-40% for both the Black-capped Chickadee (Greenwood et al., 1978; Hansen, 1978; Lawrence, 1958; Weise and Myer, 1979; Winkel, 1981) and the Tree Swallow (DeSteven, 1978). Lower rates of return are expected for birds marked as young (about 5% for both species of birds, (DeStevens, 1978; Weise and Meyer, 1979)).

The densities of breeding pairs of Chickadees and Tree Swallows may be expected, respectively, to be about 3/ha (VanVelzan, 1980) and 5/ha (Chapman, 1939). The presence of nest-boxes on the study plots will significantly increase these values within a few years because both species will use the nest-boxes in preference to natural nesting sites and will therefore aggregate on the plots.

Section 3.B.2. Small mammals. One mammal to be studied is the deermouse, Peromyscus maniculatus. It is likely to be either the most abundant or second most abundant small mammal in the forest

near the ELF Communication System (Seale et al., 1976) and is
well-known to occupy and rear young in nest-boxes (Jackson, 1953;
Nicholson, 1941; Trudeau et al., 1980).

The second species to be studied will be the northern flying
squirrel Glaucomys sabrinus. This species is likely to be abundant
[1](R. H. Baker, personal communication). If preliminary trapping
should indicate this species is not sufficiently abundant in the
forests adjacent to the ELF Communication System, we will select
another squirrel species. For instance, red squirrels (Tamiasciurus
hudsonicus) are also likely to be locally abundant. Both flying and
red squirrels are known to use nest-boxes (Muul, 1968).
A significant life-history difference between deermice and flying
red squirrels is that the deermice live beneath the snow all winter
in areas of deep snow, and they are likely to enter torpor
frequently in winter (Fuller, et al., 1969; Lynch, et al., 1978).
The squirrels, by contrast, remain above the snow and, so far as is
known, are active all the time (Pauls, 1978). Even in summer, the
squirrels are more arboreal than deermice and tend to move over a
broader area.

Reproductively mature deermice are likely to have about four
litters per year (Burt, 1957). Flying and red squirrels average
two litters per year (Burt, 1957; Sollberger, 1943).

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[1]The data of Seale et al. (1976) would not be expected to indicate
the true abundance of flying or tree squirrels because traps were
set only on the ground and most traps (93%)were too small to capture
squirrels.

The densities of breeding pairs of deermice, flying
squirrels and red squirrels may be expected, respectively,
to be about 4/ha (Blair, 1942; Manville, 1949), 2/ha (Burt, 1957;
Sollberger, 1943), and 1/ha (Burt, 1957).

Section 3.C. Test site selection

To detect effects of the ELF Communication System, if they are
present, we will compare animal attributes in test plots with those
in paired, spatially separated control plots.

Test plots will be areas in which electrical and magnetic
fields attributable to the ELF Communication System, measured
in the soil near the earth's surface, approximate 0.07 volt/
meter and 0.03 Gauss, respectively. On test plots, electric
and magnetic fields attributable to ELF sources other than the
Communication System, as measured in the soil and air near the
earth's surface, will be at least one order of magnitude lower
than those attributable to the System.

Control plots will be areas sufficiently distant from the
ELF Communication System that electric and magnetic fields
attributable to the System, measured in the soil near the earth's
surface, will be at least one order of magnitude, and preferably two
orders of magnitude, below those on test plots. Electric and
magnetic fields in air and earth attributable to ELF sources other
than the Communication System will not differ by more than one order
of magnitude between control plots and their paired test plots.
Measurements of electric and magnetic fields adequate to assure that
the above stipulations are met will be requested from IITRI as
furnished information.

For purposes of comparison and statistical analysis, each test plot will be paired with a particular control plot. The two plots of a pair will be matched as closely as possible for vegetation (species, density, maturity), soil type, drainage, exposure to sunlight, and type and abundance of standing or flowing water. Further, in the process of selecting plots, small mammals and nesting-birds will be sampled by live-trapping and visual observation to assure similarity of species composition and abundance. In subsequent discussion, paired plots will be identified as Tx and Cx (x= 1 - 5). That is, C1 will be the control plot specifically paired with test plot T1, and so forth.

Many parts of our proposed work on forest-dwelling species will be carried out on a pair of forest plots identified as T1 and C1. On these plots, activities that could artificially depress population numbers or interfere with normal behaviors will be minimized or entirely avoided. Specifically, animals will not be killed on these plots, and only very limited use will be made of live traps (the latter proviso is important because animals caught in traps are behaviorally removed from the population until released). Among the parameters to be studied on these plots are the growth and development of nestlings and behavior of both adult and nestlings.

Populations of birds and mammals are sufficiently dispersed that all plots will need to be of large size to encompass statistically adequate numbers of animals. Plots T1 and C1 will

need to be particularly large because much of the work on them will involve study of breeding pairs and their young, and the plots will need to include sufficient numbers of pairs rather than merely individuals. The size planned for each of these plots is 30 hectares.

The plots will be rectangles measuring 100 m x 3000 m. In this way, within-plot variation in distance from the ELF Communication System antenna will be minimized (see Figure 1).

Part of our effort will be directed at measuring the population density of forest-dwelling species. Carrying out these measurements on mammals will entail extensive live-trapping. Because such live-trapping is incompatible with the behavioral measures to be made on plots T1 and C1, a second set of forest plots, identified as T2 and C2, will be used for the measurements of population density. These plots will be located at least 800 m from plots T1 and C1 and will measure 100 x 1500 m, half the size of the other plots. Although measures of population density on birds will be carried out by visual observation instead of trapping, those measures will be carried out on T2 and C2 along with the measures of mammalian population density to maximize comparability of the results on birds and mammals.

Some of our experiments will involve deliberate removal of animals from plots. In particular, part of our study will involve the destruction of eggs taken from Black-capped Chickadee nests to assay for any prenatal developmental abnormalities that may occur. Other experiments will entail displacement of adults from plots to assess homing ability; animals failing to home will have been
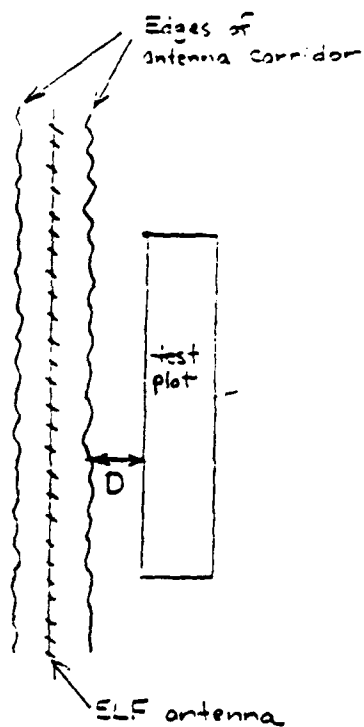
FIGURE 1. Orientation of a forest test plot in relation to the antenna (not to scale).

artificially removed from study populations. Because artificial
removals would be incompatible with the studies to be carried out on
plots T1-T2 and C1-C2, a third set of forest plots will be
established for the prenatal-developmental and homing studies.
These plots, identified as T3 and C3, will measure 100 m x 1500 m
and will be located at least 800 m from T1-T2 or C1-C2 to assure
that the removal of animals will not affect populations on the other
plots.

As noted, plots T1 to T3 and C1 to C3 will be forest plots used
for study of forest species. A significant concern is that the
corridor for the ELF Communication System antenna will constitute a
break in the forest habitat, and the proximity of the corridor
habitat to test plots T1 to T3 may itself affect bird and mammal
populations resident on the test plots by virtue of the various
types of edge effect (Gates and Gysel, 1978).

The ideal control for potential corridor effects would be to
place test plots and corresponding control plots in identical
proximity to identical corridors. Indeed, we would urge that IITRI
take steps to provide, in areas suitable for control plots,
corridors that are identical to corridors for the System antenna
(e.g., if a stretch of corridor could be freshly cleared in an area
suitable for control plots). To the extent that control corridors
identical to antenna corridors can be found or are constructed, the
distance between test plots T1 to T3 and the edge of the antenna
(D in Fig. 1) could be made as short as 10-20 m, thereby maximizing
the intensity, on test plots, of the ELF fields produced by the

antenna. If suitable control corridors cannot be found or
constructed, we will place the test plots T1 to T3 at a distance (D)
of 100-150 m from the edge of the antenna corridor. At this
distance, it is reasonable to believe that there will be no edge
effects in the test plots (Gates and Gysel, 1978).

Part of our study will be on Tree Swallows, which nest in open
habitats along edges of forests and in natural clearings. For this
part of the study, we will need test and control plots in such
habitats. We plan a test plot, T4, and a control plot, C4. The
test plot will be a segment of the antenna corridor and thus will be
particularly close to the antenna. The length of the test plot will
be 3000 m; the width will be the same as the width of the antenna
corridor (ca. 70 m). Control plot C4 will be of identical
dimensions and located in a pre-existing or newly constructed
control corridor that is as closely matched as possible to test plot
T4 in vegetational and physical characteristics.

An efficient layout from the viewpoint of day-to-day work would
be to place plot T4 in the corridor immediately adjacent to plot T1
and plot C4 adjacent to C1. This layout will be used if possible.

An additional set of test and control plots in corridors will
be necessary for the homing experiments on Tree Swallows. These
plots are designated T5 and C5 for the test and control,
respectively. The plots will be small because the number of pairs
needed for study is only 20 per plot. The T5 plot will be placed
near plot T3 if possible. C5 will be similarly placed near C3.
Both T5 and C5 (as well as T3 and C3) will be at least 800 m away
from T1 and C1, T2 and C2, and T4 and C4 plots. All plots to be used

are summarized as follows:

Forest Plots

T1 and C1 -- No deliberate killing of animals, minimal
live-trapping. Used for studies of
behavior, growth, and development.

T2 and C2 -- Extensive live-trapping of mammals. Censusing
of bird populations. Used for monitoring
population density and for studies of
physiology.

T3 and C3 -- Artificial killing and removal of animals. Used
for studies of homing and prenatal development.

Corridor Plots

T4 and C4 -- No deliberate killing, minimal live trapping.
Used for studies of behavior, growth, and
development of Tree Swallows.

T5 and C5 -- Trapping and banding of Tree Swallows. Used for
homing studies.

Some plots may be so large that it will be impossible to find
single test and control areas that, over their entire extent, are
well matched for vegetational and physical characteristics. Should
this prove to be a problem, plots may be subdivided. For instance,
T1 and C1, instead of consisting of contiguous 30 ha areas, may each
be divided into three 10 ha subplots, with the subplots being
matched in pairs. Subdivision of the plots would be disadvantageous
in that subplots would be more time consuming to reach. However,
subdivision would have statistical advantages by permitting analysis

for between-plot variation. The necessity for plot subdivision will not change our basic experimental design, and we shall continue to assume, for the discussion herein, that the plots will be contiguous areas of the dimensions specified.

All plots will be selected and surveyed during the first year of this study. As part of the survey, grids and transects as specified in later parts of this proposal will be established.

Section 3.D. Plot size justification.

Within a pair of test and control plots, the size of the plots is based upon the calculated numbers of data points required to detect, with statistical significance, relatively small differences between plots in the most variable of parameters to be measured on the plots. Plot size is therefore different for each plot set since different studies will be conducted on them.

The large size of the plot-pair T1-C1, 100 m x 3000 m, is necessary because of the expected nesting density of the Black-capped Chickadee and the required number of pairs to test for small differences in reproductive and behavioral parameters. We have used the statistics of dispersion of clutch size to estimate the required sample size as clutch-size data are available from other studies (Kluijver, 1961; Odum, 1941a, 1941b, 1942) and in our experience represent the "worst case" in variation within a parameter. Our criterion for statistical detection of effects of the ELF Communication System on the clutch size of chickadees is as follows. We wish to be 90% certain of detecting a 10% change in clutch size on T1 compared to C1 at the 0.05 level of significance.

30

A 10% difference in clutch size amounts to a 0.5 egg increase or decrease in the average clutch size. The sample size required to meet this criterion, based on a coefficient of variation of 20% (Kluijver, 1961), is about 55 pairs of nesting birds on plot T1 and on plot C1 (Sokal and Rohlf, 1969). Therefore, we plan to place nest-boxes at intervals of 50 m on two lines 100 m apart and 3000 m long on each plot. There will therefore be 60 boxes per line and 120 boxes per plot. We expect about a 50% rate of occupancy (Lack, 1954; Winkel, 1981), or 60 breeding pairs, which will allow for a small margin of extra pairs.

The requirements for plot size on T1 and C1 are the same for flying squirrels (30 ha) as for chickadees; studies of deermice also require that size. The planned number of nesting-boxes for flying squirrels on T1 and C1 is 120, or 4 boxes per ha. The expected occupancy rate is 25% or 30 pairs on each plot. This is the sample size required to be 90% certain of detecting a 20% difference in litter size at the 0.05 level of significance (Sokal and Rohlf, 1969). For the flying squirrel, we are willing to increase the percent difference to be detected between test and control plots because of the higher variability in litter size for this little studied genus (Burt, 1948, 1957; Sollberger, 1943).

Deermice will require a 15 ha subplot of T1 and C1. It is calculated that 30 pairs will be required for study on each plot. We plan to provide 120 nesting-boxes per plot, or 8 per ha. Assuming an occupancy rate of 25%, this number will provide a 90% chance of detecting a 10% difference in litter size at the 0.05

level of significance (Sokal and Rohlf, 1969). The deermice plots will therefore be consistent with the statistical criteria established for chickadees on T1 and C1.

The requirements for the size of plots T2 and C2 are based on the need for an area of adequate size to census forest birds visually and small mammals by live-trapping. Most bird censuses in forest habitat require 10 to 15 ha (Dawson, 1981; Engstrom, 1981; Ralph and Scott, 1981). This size area is also adequate for small mammals (Smith et al., 1975).

Requirements for plot size for T3 and C3 are based primarily on the need for destructive sampling of eggs from chickadee nests for the study of prenatal developmental abnormalities. Approximately 334 eggs at various ages of development will be required from each plot to be 90% certain of detecting a 4% change in the rate of abnormalities at the 0.05 level of significance (Sokal and Rohlf, 1969). It is estimated that to obtain this number of eggs will require sampling from 20 to 30 nesting pairs per plot, over a 3 to 4 year period assuming that up to 4 eggs can be taken from each nest. This will require 40 to 60 nesting-boxes per plot, assuming a 50% occupancy rate. Other bird studies on these plots, namely the homing study, will require an additional 10 pairs on each plot. Thus, the total number of nesting-boxes is 60 per plot. They will be spaced at 50 m intervals, as on plots T1 and C1. This yields 30 boxes per 1500 m line for a total of 60 boxes per plot. Small mammal homing studies on T3 and C3 will require less than the full size of the plot.

Plots T4 and C4 can be slightly smaller in area than T1 and C1 because of the social breeding behavior of the Tree Swallow (Chapman, 1939; Western, 1979). We use the same statistical requirements on these plots as for T1 and C1. Data on the variation in clutch size for Tree Swallows suggest that the sample size required to detect differences between the test and the control will be about 55 pairs of birds per plot since the coefficient of variation is similar to that found for the chickadee (Paynter, 1954). This yields a need for 120 boxes per plot, assuming an accupancy rate of 50% (Paynter, 1954). The boxes will be placed at intervals of 50 m, on lines about 20 m apart, for the length of each plot.

Plots T5 and C5 will be 70 m x 1000 m in size and will have 40 nest boxes placed in pairs about 20 m apart at intervals of 50 m. Data in the literature suggests that we can expect a 30 to 50% return rate (Southern, 1959) in the homing studies to be done on these plots. We therefore do not need sample sizes larger than 15-20 pairs of birds per year per plot.

Section 3.E. Nest box design and monitoring.

Section 3.E.1. Nest-box design: The nesting boxes described above for birds and small mammals wil be constructed to meet the specific requirements of each of the test species. Especially critical are the size of the entrance hole and the internal dimensions of the box. For Chickadees and Tree Swallows, the nest box will be a cylinder 215 mm high inside with an internal diameter of 140mm. The diameter of the entrance hole will be 29mm. For deermice, the nest box will be a square of 100mm on all internal sides. The entrance will be 25mm in diameter. For flying squirrels, the box will be a rectangle with the floor dimension of 150mm by 130mm and a height of 250mm. The entrance hole will be 40mm in diameter.

All nest boxes will be constructed from a mixture of cement and sawdust (Cohen, 1963, page 14, Figure 2). All construction will be done at Michigan State University by our work force. A mold will be constructed so that the circular chickadee and Tree Swallow nest boxes will have a unitized roof and main chamber. The front and floor will be molded separately and attached later. The front is removable to allow easy access to the inside of the nest by the investigator. Square or rectangular nest-boxes will be made with the top, sides and back as a single unit. As with the circular nest-boxes, the front will be removable.

The entrance hole of bird and mammal nest boxes will be fitted with a small tube made of plastic pipe. The tube will be about 4 cm long. The CRPID monitoring system transmitter (see Section 3.E.2) and receiver unit will be mounted on the outside of the tube. The
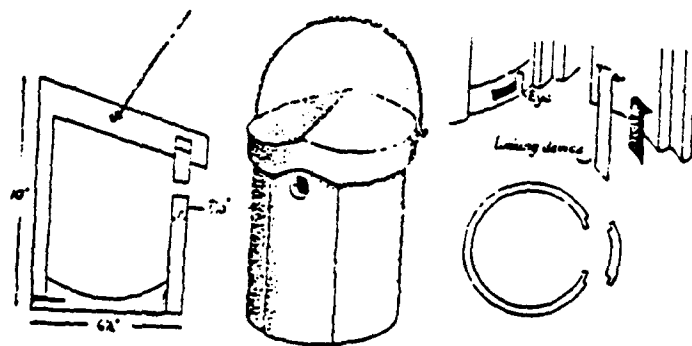
Figure 8.

A. 8. CEMENT-SAWDUST BOXES (Figure 8, Plate 1).

FIGURE 2. Design features of the cement-sawdust nest box. The one shown is for birds. The mammal nest boxes will be rectangular. Diagram is not to scale. (From Cohen, 1963)

tube will force the bird to pass close enough to the transmitter-receiver system to insure its proper operation.

The floor of the bird nest-boxes will be molded with a small hole in the center to allow the installation of the thermal eggs with a connecting wire from a thermocouple to pas to the outside to a recording devise.

This design has the advantage of simplicity of construction low cost, low maintenance, and durability in the field. In particular, woodpeckers and gnawing mammals will not be readily able to damage the entrance hole. The boxes are weather resistant and will not need replacement during the duration of the study.

Section 3.5.2 <u>Nest-box monitoring</u>

<u>Introduction</u>

Monitoring of animal movement into and out of a nest can pro-
vide many insights. Study of parental movement will indicate the
amount of time the parents spend foraging and nurturing the young.
Study of nestling movements will indicate the behavioral matura-
tion of the young. The understanding generated by study of nest-
box activity is vastly increased if it is possible to identify
movements of individual animals as well as general activity pat-
terns. Many methods have been devised to provide individual
identification, some more successful than others. In his book,
<u>Bumblebee Economics</u> (Harvard University Press, 1979, pp. 110-
114), Bernd Heinrich describes a particularly successful such
method:

> An automated beehive developed by Tracy Allen of
> Berkeley has made it possible to keep precise records of
> bees entering and leaving a hive, as well as of the
> weights of their foraging loads. Allen's hive is based
> on an electronic tag that an untiring and nearly infall-
> ible machine . . . can recognize. The electronic tagging
> system can be used to keep track of the foraging activ-
> ities of individual bumblebess, to obtain detailed infor-
> mation on the bees' time and energy budgets, and to study
> the ontogeny of foraging efficiency, division of labor,
> and other problems of foraging behavior in relation to
> resources inside and outside of the hive. The system is
> based on small tags (1 mg, 2 mm diameter) that can be
> glued onto the thorax of each bee. Each tag contains a
> tiny resonant circuit that, when energized, (emits) a
> particular resonant frequency or tone. Each tag has a
> slightly different circuit with a particular resonant
> frequency in the 500 to 1000 (mHz) range. A detector at
> the hive entrance scans the whole available frequency
> range, and if a bee passes under it, the tone emitted by
> its tag is amplified and processed for recording and com-
> puter analysis.
>   The tag emits a tone only when it is energized by a
> small transmitter, which is turned on each time a bee
> nears the detection area. The transmitter emits the
> whole range of frequencies from 500 to 1000 mHz. The

tag, being most sensitive to its own resonant frequency, ignores all the others. Thus, the tag gives off, and the receiver picks up, only one narrow band of frequencies. Of course, the whole system would be short-circuited if the receiver could hear the transmitter directly. But this is prevented by their being placed at right angles to each other.

We have met with Tracy Allen, now with the Department of Electrical Engineering at the University of California at Berkeley, and he has agreed to consult with us in the modification of his equipment for our use. A letter from to that effect, and a statement of materials and estimated costs provided by him, are attached to this section.

The design details of Allen's device have not yet been published in the open literature, but he will be giving a public presentation of his system at the Seventh International Symposium on Biotelemetry to be held at Stanford University on 15-20 August, 1982.

Continuously Recording Passive Identification Device (CRPID)

We have designated the modified Allen-design equipment that we will be using as continuously recording passive identification devices (CRPIDs) to describe their function. They will allow the continuous monitoring of all nest-box activity and will permit the identification of individual animals as they enter or leave the nest box. The equipment is described as passive because the resonant coils carried by the study animals are passive until energized by the transmitter when the animal trips the sensor at the nest-box entrance.

The nest boxes will be fitted with special entrance ways that will contain switches and circuitry to detect the presence of an animal and activate the identification equipment. In addition, the entrance ways will detect the direction of movement of the animals passing through it. When these data have been generated by the device, a signal will be sent to the data logger (Observational Systems, model OS-3) indicating that data are available for collection.

Data Collection

The data logger will then retrieve the data, format them (adding nest box ID number, date and time of collection), and store them in memory. The data logger has 16k of nonvolatile RAM available for data storage and and additional 16k of EPROM (which will be custom programmed for our data logging needs) for program storage. In addition to recording animal movement and identification data, the data logger will periodically activate and sample the data from a thermal probe within the nest box, then will format and store these data.

Data will be retrieved from the data loggers by field workers who will visit the data loggers and transfer the data from memory to cassette tape using cassette recorders supplied by Observational Systems, Inc. These cassettes will be returned to the field research laboratory where they will be entered into the dedicated microprocessor system by first reloading the data into another OS-3 data logger and then by dumping the data from the OS-3 to the microprocessor via an RS-232 connection.

Relationships to ELF Transmission

We recognize that the CRPID equipment will produce radio transmissions and that these could conceivably add to or interact with effects due to the ELF transmissions. However, we feel that the use of the CRPID equipment is justified for the following reasons. (a) Test animals will be exposed to CRPID transmission only for very short periods of time, since the CRPID system will not be transmitting except when it is triggered by the passage of an animal through the entrance way. Even assuming as many as 50 entrances and exits per animal per day, each animal would be exposed to CRPID transmission for no more than 100 seconds per day, whereas each animal on a test plot will be exposed to constant ELF transmission per day. Thus, there will be at least a three-orders-of-magnitude difference in the temporal exposure of animals to the two systems. (b) The CRPID system will transmit in the hundreds of megahertz range, whereas the ELF system will transmit at less than 100 Hz. Thus, there will be at least a five-orders-of-magnitude difference in the transmission frequencies. (c) Because CRPIDs will be used on both test and control plots, any additive CRPID effects will not influence the detection of any actual ELF-induced differences that do exist between test and control plots. (d) We will design our sampling techniques so that any interactive effects between the ELF transmissions and the CRPID transmissions can be statistically detected, should they occur. In those experiments in which CRPID equipment is used for continuous monitoring, we will equip matching numbers of nest boxes in the test and control plots with CRPID entrance

ways but without CRPID radio transmitters/receivers. Animals
entering and leaving these nest boxes will be detected by the
entrance-way switches but will not be exposed to CRPID radio
transmissions and will not be individually identified. Data from
the active CRPID devices will be collapsed to provide similar
unidentified-animal activity data, and a two-way analysis of
variance (ELF-on vs. ELF-off; CRPID-on vs. CRPID-off) will be
used on these crude data to detect any interaction between ELF
and CRPID transmission.

Section 3.F <u>Data Acquisition, Management, and Analysis</u>

<u>Introduction</u>

For a large cooperative multidisciplinary project to be suc-
cessful, care must be exercised to ensure that data are acquired,
indexed, and maintained in a manner that permits ready retrieval
and analysis. We have determined that in full operation (i.e.,
from 1983 onwards) all of our research projects together will
generate from 20 - 30 megabytes of coded data per year. A data
base of this size cannot be managed manually, if timely retrieval
and analyses are to be made. Therefore, we propose to develop
and maintain a computer-based data-management system.

We have consulted with Dr. Lewis H. Greenberg (the Director
of the Computer Laboratory at Michigan State University--see his
letter, attached) and have determined that maintaining a data
base of this size as permanent disk files on MSU's mainframe com-
puter would be prohibitively expensive. At current rates, one
year's data would incur disk storage fees in the range of $50.00
to $75.00 <u>per day</u>. By 1986, when the Michigan ELF facility is
scheduled to begin operations, the disk storage fee for all accum-
ulated data would be in the range of $73,000.00 to $109,000.00
per year, and the accumulated disk storage fees over the research
period would be in the range of $182,000.00 to $273,000.00. The
unacceptability of these expenses is even more striking when it
is recognized that these figures reflect only storage fees and do
not include any computational charges.

Besides the unacceptable expense, storing the data on the
University's main frame computer would make it difficult for work-
ers in the field to obtain timely and detailed summaries and anal-
yses. We propose to solve both of these problems by acquiring a
dedicated microcomputer that can be used on location at our re-
search site to index, store, retrieve, and analyze data. Micro-
processors are currently available that have one megabyte of ran-
dom-access memory, on-line access to 40 megabytes of data stored
on a Winchester disk drive, and the capability of archiving data
on 17 Mb cartridge tapes. With such a system available in the
field, data will be entered on a daily basis, using automated
techniques whenever possible to speed the flow of data and to
eliminate transcriptional errors. The data will be indexed by
software as entered so that relevant data subsets will be easily
assembled for statistical analysis through the specification of
the appropriate index codes. Furthermore, with such a system it
will be possible to have an entire year's data base stored on
hard disk so that the full year's data will be readily accessed
to provide timely summaries, reports, and analyses. In addition,
analyses of multi-year data sets will be performed by loading the
appropriate subsets of data from the backup tape cartridges to
the hard disk. Then the presorted data subset will either be
analyzed with the dedicated machine or disposed to the Univer-
sity's mainframe for analysis. Even if relatively low baud rates
prove to be required to permit data transmission to the mainframe
over standard telephone lines, the 40 megabyte hard disk drive
will permit the data transfer to be accomplished over night with-

out operator attention, as would be required if, for example, the
data were stored only on limited capacity floppy diskettes.

Dr. Lewis H. Greenberg, Director of the Michigan State University Computer Laboratory, has reviewed these plans and has found them to be meritorious. In addition, he has verified that our proposed system can be easily interfaced with the Michigan State University mainframe computer (see attached letter).

The Dedicated Microprocessor System

We propose to employ a dedicated microprocessor system, using the Altos model ACS8600-14 16-bit computer. This machine possesses all of the attributes outlined above and has nine RS-232 ports and one parallel port available for peripherals. The basic configuration of our proposed system is illustrated in the following figure.

Configuration of the Dedicated Microprocessor System

```
┌─────────────────────────────────┐        ┌─────────────────────────┐
│     Altos 1 Smart Terminal      │────────│                         │
└─────────────────────────────────┘        │                         │
                                            │                         │
┌─────────────────────────────────┐        │                         │
│     mark-sense forms reader     │────────│                         │
└─────────────────────────────────┘        │                         │
                                            │                         │
┌─────────────────────────────────┐        │                         │
│        Anderson Jacobson         │        │                         │
│      variable-rate modem         │────────│                         │
└─────────────────────────────────┘        │   Altos ACS 8600-14     │
                                            │       Computer          │
┌─────────────────────────────────┐        │                         │
│  Observational Systems Data      │        │                         │
│  Transcriber for model OS-3      │────────│                         │
│  data logger                     │        │                         │
└─────────────────────────────────┘        │                         │
                                            │                         │
┌─────────────────────────────────┐        │                         │
│      Epson MX-100 printer        │────────│                         │
└─────────────────────────────────┘        │                         │
                                            │                         │
┌─────────────────────────────────┐        │                         │
│   MTU-3 cartridge tape drive     │────────│                         │
└─────────────────────────────────┘        └─────────────────────────┘
```
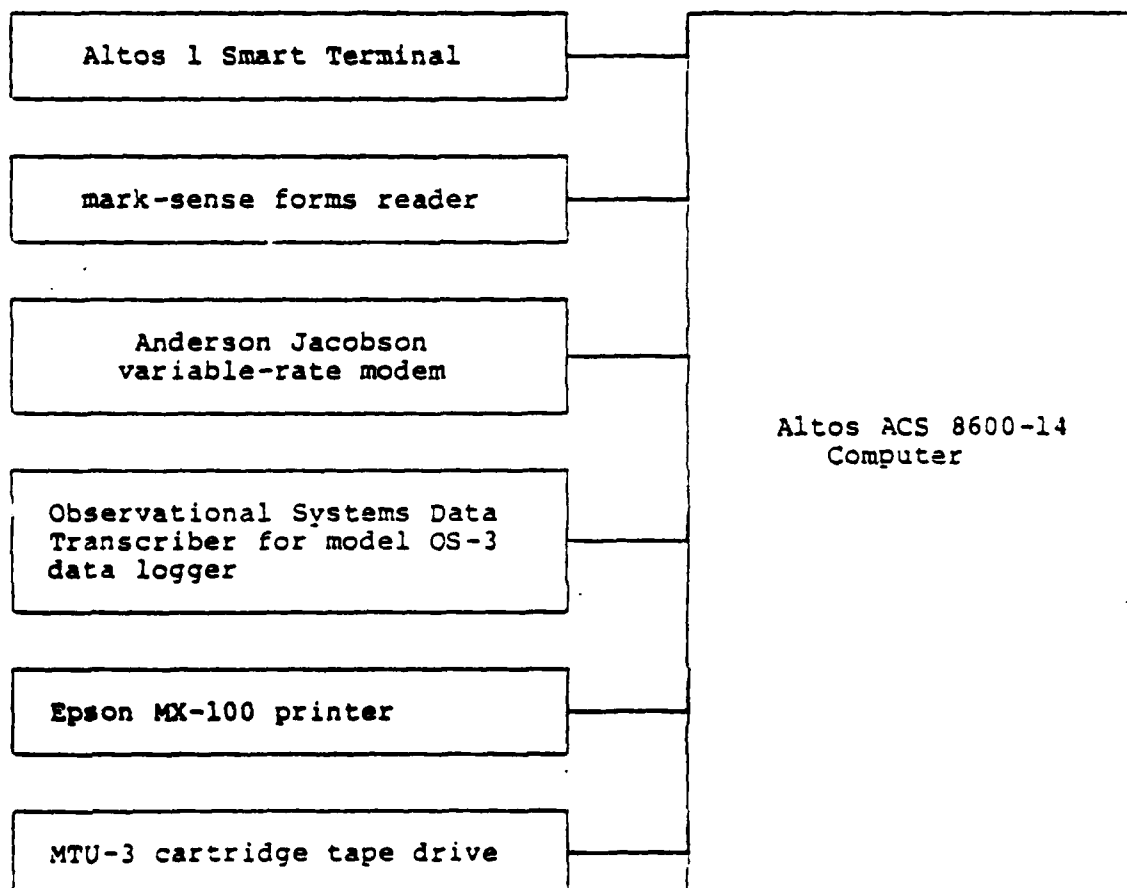
Data Acquisition

We propose to facilitate data acquisition while simultaneous-
ly minimizing transcription errors through the use of automated
data-entry techniques whenever possible. As it is generated in
the field, data from the continuously recording passive identifi-
cation (CRPID) equipment (cf. Section 3.E.2 of Part 1 of the Tech-
nical Volume) will be stored automatically in Obervational Sys-
tems model OS-3 data loggers. The data will be retrieved by
field workers (by copying onto cassette tape in a field cassette
recorder also supplied by Observational Systems), transported to
the field research facility, and entered into the dedicated micro-
processor by first reentering the data into another OS-3 data
logger that communicates with the dedicated microprocessor via an
RS-232 port. All manually acquired data, such as population den-
sity information, growth rates, etc., will be entered, immediate-
ly as acquired, on mark-sense forms which will then be read into
the microprocessor. The use of mark-sense forms for initial data
recording will eliminate both the time-consuming manual reentry
of the data into the microprocessor and the concomitant risk of
transcription error associated with manual reentry of data. Fur-
thermore, the mark-sense forms themselves will provide an addi-
tional machine-readable safety backup system for data storage.

The data will be entered by accessing an interactive software
system that will query the operator regarding the data being
entered and then will assign appropriate index values to the data
and will store it on disk in a standardized, indexed format. The
use of software-driven data-storage routines will ensure that all

equivalent data are indexed and stored in equivalent formats so that retrieval and analysis can be readily performed in future years, even if personnel changes have occurred. The software will be fully trapped, so that incorrect or meaningless entries by the operator will not result in damage to or loss of the data base. Dr. Robbins, the senior scientist in charge of data-base management, has had considerable experience in developing interactive data-storage and retrieval software and currently is operating such a system on the University mainframe computer.

Data Management

In addition to the software-driven indexing of data that will occur on data entry, quality control of data management will be achieved through a system of checks and balances in which all newly entered data (whether automatically or manually obtained) will be examined to detect outliers through comparison with appropriate running and seasonal means and variances. All data points that are more than three standard deviations from their reference means will be flagged so that manual checks may be made to determine if they are valid values or if a machine or human error has occurred.

For safety, all data will be stored in at least two media. All mark-sense data forms from manual entry of field data will be indexed and stored. All data entered onto the hard disk of the dedicated microprocessor will be immediately copied onto a floppy diskette for temporary storage. On a regular basis, data will be copied from the hard disk to cassette tapes for archival storage. Archivally stored data will not be maintained in the same physical location as the dedicated microprocessor.

To ensure that the field workers are informed as to the progress of their work, summary reports and analyses of the data base will be compiled on a monthly (and in some cases weekly) basis. To permit the development of the most efficient form of data indexing, considerable effort will be devoted to anticipating the summary report and analysis needs of the field workers prior to the installation of the system in the field.

Data Analysis

Of course, the ultimate goal of our research, and thus of our analytical techniques, will be the paired comparison of measures taken on control plots with those taken on test plots while the ELF System is active. However, we will be collecting and analyzing data from the test and the control plots prior to the activation of the ELF System.

These pre-transmission data will serve several purposes: (a) They will permit a determination of the intraplot variances associated with all of our experimental measures allowing experimental designs and planned sample sizes to be adjusted prior to the activation of the ELF antenna so that maximal information may be extracted from the statistical analysis of the data obtained after the activation of the antenna. (b) They will allow us to determine the degree, if any, of consistent interplot variation that exists prior to the activation of the antenna, thereby permitting us to make allowances for this preexisting variation when analyzing the data collected after the activation of the antenna. (c) They will allow us to determine the degree, if any, to which our selected measures are correlated with weather or other am-

bient conditions, thereby permitting us to increase the statisti-
cal power of our analysis of the data collected after the activa-
tion of the antenna through the use of standardization procedures
or the use of analysis of covariance. (d) They will provide pre-
antenna-activation data for the test plots, thereby permitting us
to make additional determinations of the effect of ELF transmis-
sion by comparing the pre-transmission data with the post-trans-
mission data. (Because this type of pre/post comparison con-
founds transmission effects with year-to-year differences, it is
not suitable as a primary means of analysis. However, it can be
useful as a supplementary analysis--especially when factorial
techniques are employed so that a significant pre/post effect
occurring only on the test plot would be detected by the occur-
rence of an interaction between ELF effects and year effects.)

For our actual statistical analyses, we plan to use one-way
(test plots vs. control plots) and two-way (test plots vs. con-
trol plots; pre-transmission vs. post-transmission) analysis of
variance to examine single-variate continuous data. For an
analysis of multivariate data (such as that involved in comparing
the growth curves of young from the test and control plots) we
will use Hotelling's T^2 test. Discrete data will be analyzed
using Chi-squared analysis.

SCHEDULE OF ACTIVITIES FOR DATA-BASE MANAGEMENT

7/82	8/82	9/82	10/82

----acquire basic computer equipment---->.

.--------begin developing software------->
.--------for data analysis--------------->

11/82	12/82	1/83	2/83	3/83	4/83	5/83	6/83	7/83	8/83	9/83	10/83

----continue software---->.
----development----------->.
.-install &-->.
.-test in---->.
.-field------>.

.--------acquire data in the field------->

.--------provide weekly and monthly-------
.--------data summaries & analyses------->

.--------ongoing software testing,------->
.--------modification, & development----->

11/83	12/83	1/84	2/84	3/84	4/84	5/84	6/84	7/84	8/84	9/84	10/84

----------------------------data reduction and analysis--------------------->

----------------------------continuous data acquisition---------------------->

----------------ongoing software modification, testing, and development----------->

----------------provide weekly and monthly data summaries and reports----------->

11/84	12/84	1/85	2/85	3/85	4/85	5/85	6/85	7/85	8/85	9/85	10/85

----------------------------data reduction and analysis--------------------->

----------------------------continuous data acquisition---------------------->

----------------ongoing software modification, testing, and development----------->

----------------provide weekly and monthly data summaries and reports----------->

SCHEDULE OF ACTIVITIES FOR DATA-BASE MANAGEMENT (cont.)

11/85	12/85	1/86	2/86	3/86	4/86	5/86	6/86	7/86	8/86	9/86	10/8
.	.	.	.	.	.	.	.	.	.	.	
------------------------------data reduction and analysis---------------------------->											
.	.	.	.	.	.	.	.	.	.	.	
------------------------------continuous data acquisition---------------------------->											
.	.	.	.	.	.	.	.	.	.	.	
----------------ongoing software modification, testing, and development------------>											
.	.	.	.	.	.	.	.	.	.	.	
----------------provide weekly and monthly data summaries and reports------------>											
.	.	.	.	.	.	.	.	.	.	.	

Section 4.   ACTUAL EXPERIMENTAL STUDIES

All of the studies discussed below will be conducted on the plots specified in Section 3.B. They are designed to answer the question outlined in Section 3.A.

Section 4.A. Demography: Do the electrical and magnetic fields of the ELF Communication System alter the population density, species composition or distribution of birds or mammals?

Section 4.A.1. Birds. Censuses of all species of nesting birds on T2 and C2 plots will be conducted twice a week starting in the last week of May and continuing through the last week of June. This time period will yield 10 censuses per plot, a sufficient sample size for computing population densities and statistics of dispersion (Ralph and Scott, 1981). The census procedure will follow the spot-map method as modified by Franzreb (1981). Censuses will be conducted in the early morning hours from 0600 to 1000. Two lines, each 1500 m long, with stations 100 m apart (total of 3000 m and 30 stations) will be censused. The estimated width of coverage along the route of the census is 50 m to either side of the census path so that a swath 100 m wide and 1500 m long on each line will be censused. The total area censused on each of the two plots is calculated to be 30 ha.

Tree Swallow plots (T4 and C4) will be censused for open habitat species of birds. The procedure will be the same as mentioned above, but the area available to sample will be smaller.

Specifically, the width of the route will be about 70 m. A single line will be established between the two nest box lines and it will have stations every 100 m, as on plots T2 and C2. Thus, 30 stations will be sampled with an estimated area of coverage of 21 ha (70 x 3000 m).

Section 4.A.2. Small Mammals. Censuses of all small mammal species, squirrel-sized and smaller will also be conducted on plots T2 and C2. Censuses will start in May and will continue monthly through October. Censusing will not be done in the winter due to the heavy snow cover present from 1 November to 1 May. Density estimates of small mammal populations will be determined by live-trapping. We will follow the techniques described by O'Farrell et al. (1977). Since the measurement of density and species composition is the primary objective of this section of the study, we will use census lines along with assessment lines. This dual approach will yield basic population parameters for less labor and materials than a grid with assessment lines only (O'Farrell, et al., 1977; O'Farrell and Austin, 1978).

In the experimental and control plots (T2 and C2), two 585 m long parallel lines 50 m apart and containing 40 trap stations each will be established. Six 255 m long assessment lines of 18 trap stations each will intersect the census lines at a 45 degree angle. Trap stations on the assessment lines will be 15 m apart. At each trap station on both the census and assessment lines there will be placed one mouse-sized and one squirrel-sized live-trap. Trapping will be conducted on eight nights beginning in the second week of each month from May through October. The census lines will

be trapped for five nights followed by three nights of trapping on the assessment lines. Traps will be baited with rolled-oats and peanut butter. Traps will be checked twice daily, once in the morning and once in the evening; the morning check will reveal captured nocturnal mammals where as the evening check will reveal diurnal ones. All animals trapped will be marked, their sex determined and released at the point of capture. The calculation of monthly densities from the live-trapping data will follow the method of O'Farrell et al. (1977).

Section 4.B. Orientation and homing: Does the electrical and magnetic fields of the ELF Communication System alter orientation and homing ability?

Section 4.B.1. **Birds.** Previous studies have shown that members of the swallow family are extremely adept at homing to the nest in conditions that probably require use of magnetic field orientation (Southern, 1959). Black-capped Chickadees are much less adept or less prone to home when displaced (Weise and Meyer, 1979; Odum, 1941c). We will test the homing abilities of each of these species using a similar technique.

In the first full year of study (1983), a set of eight nesting pairs of each species will be selected for study on each of the experimental and control plots (T3 and C3 for chickadees and T5 and C5 for Tree Swallows). Females will be used for the homing study because they are more highly motivated to return to the nest than males (Southern, 1959). The female member of each pair selected for study will be captured early in egg laying or incubation (only the female incubates eggs in tree swallows, Bent, 1942; Western, 1979;

both male and female incubate egg in the Chickadee, Bent, 1946) and fitted with a small (1mg) tuned coil to be used for detecting her presence when and if she returns after being displaced. Each female will also be fitted with a Fish and Wildlife Service band and a colored leg band. The nest box of each of these pairs will be fitted with a special continuous recording passive identifying device (CRPID-- described in Section 3.E.2.) several days prior to the experiment. The CRPID system will allow us to determine the precise time of return of the female to the nest. The fact that the sexes of both Tree Swallow and Black-capped Chickadee are indistinguishable by plumage characters makes it imperative that we use individual markings to note when the female returns. The use of the CRPID system will allow us to monitor more nest boxes with far fewer personnel than we could otherwise afford since the only other option would be for a person to be stationed continuously at a box until the bird returned or we decided that the bird would not return.

On a test day, previously marked female Tree Swallows or Black-capped Chickadees will be captured at the nest in the early evening following the technique of Southern (1959). We will select test days with overcast skies, to the extent possible, since the reduced visibility of celestial cues will increase the probability that the birds will use magnetic field orientation in order to home to the nest. A total of four birds from the test and control plots will be used per day of the experiment. There will be four release points per plot, one at each of the cardinal directions from the center of the plot. Two release sites will be selected randomly from the four on the first test. The remaining two release points will be used in the next test, and so forth.

The release sites will be specific to each plot since the plots will be at least 800 m, and probably further, from each other. That is, birds captured on T4 will be released from points equidistant from its mid-point. Birds captured on C4 will be released from a second set of points equidistant from its mid-point, and at the same straight-line distance as the release points for T4 are from the center of T4. For Tree Swallows, release points will be about 100 km from the mid-points of the plots.

Black-capped Chickadees will be tested similarly to the Tree Swallows, but the release distance will be 2-4 km. Studies by Odum (1941c) have shown that this species is capable of homing these short distances. All chickadees studied in the homing experiment will be taken from plots T3 and C3. In each year of the study, as with Tree Swallows, four individuals will be released from each of the four cardinal points of release.

In tests with either species, the CRPID system (see Section 3.E.2.) will be used to monitor the nest box of displaced birds for two days after release, or until the bird has been recorded as returned to the nest, whichever is shorter.

The results of the homing studies in the first year will be used to alter the design of subsequent year's studies, if appropriate. Possible additions or alterations in the design include increasing the distance the birds are displaced from their nest and number of directions of release from the nest site, and/or larger numbers of releases on the same night when the weather conditions favor maximal use of magnetic field orientation for homing. Furthermore, if Black-capped Chickadees do not prove to be

adequately capable of homing, they will be eliminated from the experiment in future years and the effort on the Tree Swallows expanded. If alterations or additions (or both) to the homing experiments become appropriate, we will revise the study plan and budget accordingly in future years.

A second aspect of homing will also be examined. This is the tendency of birds to return to the area of the previous year's breeding, a behavior that has been termed site fidelity. Both Tree Swallows and Black-capped Chickadees show this pattern, and we plan to test for effects of ELF on site fidelity by measuring the average distance between nesting locations from year to year. This study will require marking each male and female nesting on the plots T1 and C1 for chickadees and T4 and C4 for Tree Swallows. We will capture them at the nest site and band them with a Fish and Wildlife Service leg band. Males can be fairly easily captured early in the nesting cycle by using tape recorded play back of territorial songs to lure them into mist nets. Females can be captured at the nest during egg laying and incubation. Males not captured early in the season can be captured during feeding of the young, although capturing them at that time is somewhat more stressful and will be avoided if possible. We will attempt to capture all nesting birds every year on plots T1, C1, T4, and C4. Recaptures and their nesting location will be noted. Young birds produced during the nesting season will be similarly marked before they leave the nest. We expect to mark up to 60 pairs of adults and their nestlings on plots T1 and C1 (Chickadees) and a similar number on T4 and C4 (Tree Swallows).

Section 4.B.2. Mammals. Although it is clear that many small mammals are capable of homing successfully, the operative cues and behavioral responses involved in the process remain speculative compared with birds (Bovet, 1978; Anderson et al., 1977). In most studies using small mammals, homing success is measured by determining the proportion of animals that return home within a given time, or by measuring the latency to return home from a given displacement distance. We will test the homing ability of the deermouse using similar techniques to these. We choose to test the homing ability of only the deermouse because the phenomenon is more clearly documented for Peromyscus than for squirrels. However, if we can show that our squirrel test species is capable of homing in pilot tests, we will expand the homing experiment to include it in future years.

Animals to be tested for homing ability will be live-trapped from test plot T3 and control plot C3. Each animal to be tested will be fitted with a mouse-sized radiotransmitter (see Section 4.E.2.) and released at the point of capture so that its activities can be monitored prior to the test. This is necessary in order to determine the animal's home range so that we will know the limits of the area to monitor for the return of the test individuals. Radiotelemetry will be used because it is not subject to the biases of live-trapping. Twelve radio-collared deermice will be monitored simultaneously per plot for 7 days which is required to determine their home ranges. From experience we know that twelve deermice can easily be radio-tracked simultaneously each night. On each of three successive mornings

(day 3-10), a different group of four radio-collared mice will be located at their nests and transferred to a holding cage preparatory to their displacement and release later in the evening. Locating radio-collared mice in their daytime nests is easily done (Rawson and Hartline, 1964; B. Ormiston, personal communication). Each of the four deermice in a group will be displaced singly approximately 200 m from the center of their home range (calculated from previous radiotracking data by using a non-parametric method of estimation (Anderson, 1982)). The four mice will be displaced, respectively, to the four cardinal points of the compass. Bovet (1972), Griffo (1961) and Cooke and Terman (1977) indicate that deermice home very successfully from 200 m. Our design assures that homing mice will be exposed to electric and magnetic fields which parallel and are perpendicular to their homing paths, as is the case for homing birds in the experiment described above. The release will occur in the late evening (2200-2400 hrs), and the home range of each individual will be monitored until dawn and again all the following night or until a return is recorded. Griffo (1961) has determined that a high percentage of returns will occur in the first two nights after displacement. In each year we expect to test approximately 36 mice in each of the plots (T3 and C3). As with the case for birds, the results of the homing studies in the first year will be used to alter the design of subsequent years' studies if appropriate.

Section 4.C. Parental Behavior: Do the electrical and magnetic fields of the ELF Communication System alter parental behavior?

Specifically, do they alter the time parent birds spend brooding, incubating and feeding their young, and the time parent mammals spend nursing and warming their young?

Of concern here is the normal pattern of parental activity in birds and small mammals on their territories and home ranges during the breeding season. If electric and magnetic fields within the strength and pattern of those produced by the ELF Communciations System are aversive stimuli or modify behavior in some way, the response of birds and mammals may well be manifested as changed normal parental behavior, such as incubating and feeding or nursing patterns.

Section 4.C.1. Birds. Incubation and brooding of eggs will be monitored with automatic temperature recording equipment. Five nests selected at random from the nests monitored during egg laying on each of plots T1, T4 and C1, C4 (total of 10 Tree Swallow nests and 10 Black-capped Chickadee nests) will be studied intensively. These nests will not be used in the study of ontogeny of nestlings detailed in Section 4.D. Disturbance by the investigators will be kept to an absolute minimum at these nests in order to preserve, as much as possible, the normal parental activities. However, we will briefly capture both of the parents associated with each nest before or during egg laying in order to fit them with the tuned coil necessary for CRPID detection (see Section 3.E.2.). Each nest will be equipped with an artificial egg in which will be placed a thermocouple. The thermocouple system will be connected to an analog to digital converter which will then enter the temperature value into an OS-3 data logger (see Section

3.F.). The OS-3 will append the time of the reading to the temperature and store it in its memory for later retreival. The temperature in the artificial egg will be recorded at intervals of every 3 minutes all day and night. Incubation and/or brooding of young will appear as elevated temperatures at least up to the age that the young are capable of thermoregulation (about 4 or 5 days after hatching -- Hill and Beaver, 1982). Brooding by the parents usually ends at this age as well. Monitoring will be carried out for a period of about 15 days of incubation and 5 days of nestling life for each of the 5 nests on a plot. In addition to monitoring temperatures as an indication of incubation and brooding rhythms, the CRPID system sensors at the nest entrance will record the comings and goings of adults. The signals received by the CRPID system will be encoded by an OS-3 data logger which will attach a time to each event.

The rate of feeding the nestlings will be monitored by recording the frequency of the adults' coming to the nest for the 5 nests on each plot mentioned above. Adult comings and goings will be monitored by use of the CRPID system. A CRPID unit will be operated at each of the five nests on a given plot for the entire period of feeding nestlings, about 15 days after hatching.

Section 4.C.2. Small mammals. Parental behavior of breeding squirrels and deermice that nest in the provided nesting-boxes will be monitored in the following manner. A subset of nesting-boxes in which pregnant or lactating females are found will be fitted with the CRPID monitoring system, and the females themselves will be outfitted (subcutaneously) with small tuned

coils weighing about 1mg). This will allow us to determine the temporal patterns of nest visitations by the female in contrast to visitations by other animals, such as the male. Monitoring will start at the time of birth, or soon after, and continue until the litter is weaned or leaves the nest permanently. As in the bird study using the CRPID system, the data output will be recorded using an OS-3 automatic data logging device.

Section 4.D. **Maturation** **of** **nestlings:** Do the electrical and magnetic fields of the ELF Communication System alter the maturation of nestlings?

Section 4.D.1. **Birds.** This study will require regular daily monitoring of nests not used in the intensive monitoring detailed above. The interval of egg laying, egg weight, hatching sequence and success, weight of the nestlings, growth of the wings and legs, the day of eye opening, the development of feathers, and date and success of fledging will be recorded (Barth, 1955; Flegg and Cox, 1977; Huggins, 1941; O'Connor, 1975; Paynter, 1954; Ricklefs, 1968; Royama, 1966; Stoner, 1945). This study will be conducted on plots T1, C1, T3 and C3 on an expected set of 20 nesting pairs in each year on each plot (for a total of 40 pairs of chickadees and 40 pairs of Tree Swallows).

Interval **of** **egg** **laying** **and** **egg** **weight:** The interval of egg laying will be recorded by simply counting eggs and keeping track of the appearance of new eggs in the nest. Both Tree Swallows and chickadees are known to lay one egg per day, usually in the early

accurate to 0.05mm, (3) the age in days at opening of the eyes (we define eye opening as the time when the eye-lids are clearly separated and the eye shows through the opening), and (4) the age when feather development occurs in the mid-spinal tract, the outer primaries of the wing, and the central rectrices. We define the start of development as occurring when the feather sheath attains 0.5mm as measured with a dial caliper. Thereafter, we will record the daily increase in length in the feathers in these localtions.

Young will be marked for individual identification with a small, colored, thread tied loosly on the leg above the foot following the technique of Oniki, (1981) up to about 6 days of age. At that age, they will be banded with a standard Fish and Wildlife Service band.

Age of fledging: The age when the nestlings leave the nest will be recorded. It is well known that disturbances near the end of the nestling period can cause premature fledging (Lack, 1966). We will, accordingly, not open the nest box or handle the young after the 14th day from hatching. Rather, all observations will be made by looking in the nest box entrance with a small mirror and battery-powered light apparatus. The number of young present will be counted if possible (the nestlings usually huddle on the nest floor and can be difficult to count). If young birds are seen outside the nest, we will conduct a visual search of the immediate area using binoculars to locate all those that have left the nest before attempting to count those still in the nest. The average age of fledging will be estimated as the time from the last hatching to the last nesting leaving the nest.

Section 4.D.2. Small mammals. Growth of the young, age at eye
opening, age at eruption of lower incisors, age at openint of the
external auditory meatus: From reports in the literature, it is
known that the breeding season for deermice commences in March and
continues through late September (Burt, 1948; Burt, 1957; Blair,
1942; and Manville, 1949). During this season, an adult female may
give birth to 4 or more litters (Burt, 1948; Burt, 1957). The
breeding season for the northern flying squirrel is split, with
the first litter born in April or May and the second born in August
(Burt, 1948; Burt, 1957).

The post-natal growth and development of young squirrels and
deermice reared in nest-boxes will be monitored on plots T1 and C1
according to the following procedure. Nest-boxes will be checked
every two days commencing with the start of the breeding season.
Nest-boxes will not be monitored daily as with birds because it is
suspected that more frequent checking may disturb breeding females
and cause abandonment of the litter (J. King, personal
communication). Nest-boxes with litters present will be examined,
and data from each nestling regarding its identity (all mice will be
marked with an identifying number at first capture or shortly after
birth), weight, age at eye-opening, age at opening of the external
auditory meatus, and age at the eruption of the lower incisors will
be recorded on mark-sense forms (see Section 3.F.) in the field. We
define eye opening as the time when the eye-lids are clearly
separated and the eye shows through the opening. Nestling weight
will be measured with a small pesola field scale accurate to 0.1gm.
Any observations of postnatal mortality will also be noted.

Age at first departure from the nest, age at weaning, and duration of excursions from the nest: Monitoring of nest-boxes for behavioral development in nestings will be recorded by monitoring movements of nestlings to and from the nest, and by determining the age of nestlings at weaning. Movements of nestlings to and from the nest will be measured in the same manner as for the parents by monitoring nest-boxes with the CRPID system (see Section 3.E.2.) This system will allow us to monitor the frequency and duration of temporary trips away from the nest-box as well as the age at weaning.

Section 4.E. Activity patterns: Do the electrical and magnetic fields of the ELF Communication System alter the day/night activity schedule of adults?

Section 4.E.1. Birds. The study of day/night activity schedules of birds was detailed earlier in Section 4.C.1. It will not be repeated here.

Section 4.E.2. Small Mammals. We propose to study free-ranging deermice in plots T3 and C3 by using radiotelemetry techniques. Radiotelemetry techniques are highly preferable to grid trapping for purposes of determining patterns of space use and activity (Amlaner and MacDonald, 1980). Complete records of animal movement can be obtained and the activities surmised based on location and other parameters, such as habitat.

Deermice will be trapped, marked, and their sex, reproductive condition and weight recorded. Adult deermice
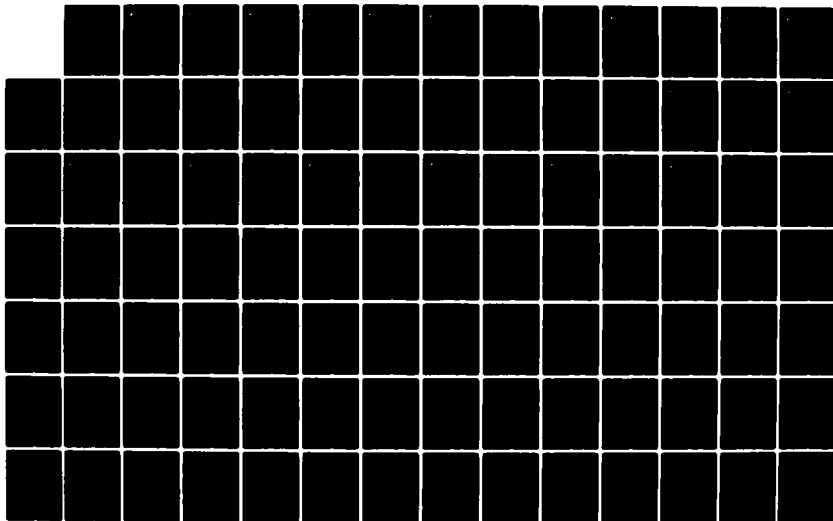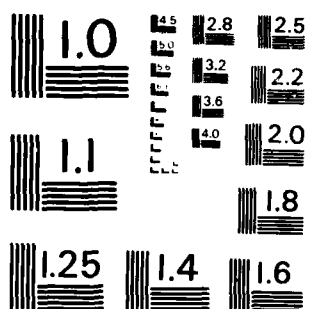
MICROCOPY RESOLUTION TEST CHART

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will be fitted with AVM SM1 Mouse Style radiotransmitters
following standard collaring procedures (Madison, 1977; Mineau
and Madison, 1977; Murphy and Gidner, 1982). A total of 36
mice each on T3 and C3 will be monitored in each year.
Twelve deermice will be radiotracked for a seven day period as
part of the homing experiment (see Section 4.B.2.). Two researchers,
each using a portable radio receiver and hand-held Yagi antenna,
will determine up to six locations for each animal per night at
roughly two hour intervals. From this spatial and temporal
distribution of locations, space use patterns, based on movement
frequencies and movement distances will be determined following the
procedures of Webster and Brooks (1981), Hermann (1977) and Murphy
and Gidner (1982).

One potential problem may arise while using radiotelemetry. We
recognize that the transmitters may emit electric and magnetic
fields. These may in some way enhance or reduce the effects of the
electric and magnetic field produced by the ELF Communication System
when it becomes operational. We may be able to estimate the
potential of the radiotransmitters to produce these fields given we
can determine the field strength produced by one in operation. We
have asked the engineers at AVM Instrument Company (Dublin,
California), a radiotelemetry equipment company, to investigate the
electric and magnetic fields produced by the transmitters. Their
preliminary calculations indicate that their SM1 Mouse Style
radiotransmitter produces electric fields about two orders of
magnitude less than the expected field produced by ELF (0.000828
volts/m versus 0.07 volts/m). In addition, the frequency of

radiotransmitter-generated signals is seven orders of magnitude greater than that produced by the ELF Communication System. For these reasons, and the fact that the level produced by the radiotransmitter is in the acceptable range for control plots, we believe that radiotelemetry can be used without biasing the over all experiment. We do not at this time have estimates of the magnetic field strength produced by the radiotransmitter. We propose therefore to use radiotelemetry to monitor the behavior of deermice contingent upon: (1) on-site testing and verification of electromagnetic fields attributable to AVM radiotransmitters, and (2) the operating efficacy of radiotelemetry equipment under the influence of a pre-existing ELF facility in Wisconsin.

Section 4.F. Fecundity: Do the electrical and magnetic fields produced by the ELF Communication System alter fecundity?

In this section of the study, we examine aspects of the fecundity of birds and mammals on the test (T1 and T4) and control (C1 and C4) plots.

Section 4.F.1. Birds: Clutch size, survival of eggs to hatching, and survival of nestlings to fledging: Data on the size of the clutch, survival of eggs to hatching, and survival of nestlings to fledging will be obtained as a part of the daily monitoring of nest-boxes in the study on maturation of young in Section 4.D. We therefore do not repeat the procedures here. It is important to restate the fact that the size of the sample generated each year in this aspect of the study will be sufficient

to show differences between plots of as little as 10% in the size of the clutch. Thus, when the antenna system is finally made operational, we will have a large baseline data set, and additionally be able to test for effects in the first breeding season.

Section 4.F.2. **Small mammals:** Litter size, survival of nestlings, gestation. Data on litter size, and survivorship to weaning will be collected during nest-box checks made every other day beginning with the start of the breeding season. As in studies on birds, sufficient data will be collected to detect differences in litter size of as small as 10% between plots. By knowing the age and identity of animals using nest-boxes, we will also be able to estimate the age at first gestation of some individuals. This critical demographic parameter will be compared between test and control plots.

Section 4.G.   Developmental Abnormalities: Does chronic exposure to
the electric and magnetic fields of the ELF Communication System
induce developmental abnormalities in resident birds?

Does it alter the occurrence of prenatal developmental
abnormalities: All studies on development will be conducted on plots
T3 and C3 using the Black-capped Chickadee.   In the first part of the
study, we will need to obtain eggs of known incubation times in order
to screen embryos for developmental abnormalities.   We assume that
the natural rate of abnormalities is about 1% (we can find no data on
this in the literature), a similar level to that observed for the
domestic chicken (Asher, personal observations).   In order to be 90%
certain of detecting an increase of 4% in the rate of abnormalities,
an increase we feel would be reasonable to detect and significant (at
the 0.05 level of significance) in terms of its effect on the
population, we calculate that we will need to collect 334 embryos per
plot.   This is not feasible to do in a single year of study, but if
we assume that the rate of abnormalities is unvarying over years, we
can accumulate samples over three years to meet the sample size
requirement.   The design will require that an additional three years
of sampling be conducted after the start of operation of the ELF
system.   We therefore plan to monitor 10 nests on each plot in the
first year of study, and from these remove a maximum of 4 eggs per
nest, for a total of 40 eggs per plot.   In future years of the
study, we will have to increase the number of nests used for egg
collections to 30 to reach our sample size requirement.   Normal
clutch size is about 7 eggs (Odum, 1941a), so that the eggs left

should insure that the parents continue incubation. It should be noted that in a study of the Great Tit (Parus major) in Europe by Kluijver (1971) where clutch size was artifically reduced by removing eggs after incubation had started (so that no more eggs were laid), the subsequent survival of the smaller clutches was greately enhanced due to lessened competition between nestlings for food. Population size on the study area increased dramatically in each year of the study. We therefore do not expect the removal of eggs to be detrimental to the population in subsequent years. Still, it will be our approach to minimize any possible effects of egg removal by spreading the collection of eggs over three years.

Does ELF alter developmental rates: A critical aspect of the study is to know the age of the embryos within a day so that comparisons of growth rates may be made between the plots and published works on other species, and so that organ development in the early stages can be described. It is in the early stages of organ formation that most environmentally induced abnormalities are expected. Staging of embryos will require knowledge of the onset of incubation. Chickadees do not start incubation until completion of the clutch so that a temperature monitor placed in the nest may be used to detect the start of warming of the eggs. An artifical egg with an implanted thermistor will be placed in nests to be monitored and connected to a Rustrak strip-chart recorder. The strip-chart will be checked daily for evidence of the onset of incubation which will be indicated by an elevated and steady temperature. The only relevant information to be recorded by the strip-chart recorder

is the exact time and date incubation begins.

Eggs will be randomly collected in such a way to insure that there will be 4 eggs at each age of incubation from 1 to 14 days. Embryos will be removed from the egg in the field and preserved in Bouins fluid, (except for some very early aged embryos that will be preserved in gluteraldehyde for later electron microscopic studies), weighed and processed for further study. Embryos up to a few days of age will be dehydrated in a graded series of ethanol to 100% ethanol, stained with Grenacher's carmine, and whole mounted. Whole mounts will be studied microscopically and photographically to determine the early developmental patterns. Older embryos will be studied for their gross morphological development. This will include measurements of _in ovo_ growth rates. Since no published data could be found for normal _in ovo_ development rates for the chickadee, this will be one of the first tasks to accomplish.

Once we have adequately described the developmental rates of chickadee embryos, we will intensify our collection of very early stages of embryos in order to screen more thoroughly for developmental abnormalities. Depending upon the rapidity of development, we will collect embryos at 1/2 to 1/4 day intervals during these very early stages of development. During the first two to three days of development, all of the major organ systems are sensitive to environmental insults. By intensifying our study of these early ages, we should be able to recognize small but significant developmental abnormalities. Section 4.H. Thermoregulation: Do the electrical and magnetic fields of the ELF Communication System alter thermoregulatory capabilities in birds and mammals?

As explained in Section 2 (pp. 12-15), the ability of animals to resist the extreme cold of winter will be assessed holistically by measuring maximal rates of heat production and the magnitudes of the fat stores accumulated during the active phase of each day. The size of an animal's fat store indicates how much fuel is available to meet thermoregulatory and other metabolic demands. The animal's peak rate of heat production indicates how rapidly fuel can be used to produce heat for thermoregulation; it also provides an index of metabolic endurance.

Peak rate of heat production: The animal's peak rate of heat production will be determined by measuring its aerobic capacity, that is, its peak rate of oxygen consumption. As is well established (Brown and Brengelmann, 1965), aerobic heat production can be calculated from oxygen consumption with good accuracy. Anaerobic heat production can be disregarded because it cannot be sustained for very long; thermoregulation, being a sustained activity, must rely on aerobic heat production, which can be sustained indefinitely.

Animals to be tested will be captured on plots T2 and C2. Deermice and squirrels will be captured using live traps. Chickadees will be captured by establishing temporary feeding stations and using mist nets to ensnare birds attracted to the stations. Traps and nets will be monitored frequently so that animals will be picked up soon after capture. The animals will then be taken to the laboratory, tested, returned to the point of

capture, and released. The physiological measurements will require only 2-3hr. Thus, animals will be removed from their natural environment for only several hours. Animals will be fed while in captivity to minimize the impact on their daily food acquisition.

The peak rate of oxygen consumption will be determined using an atmosphere of 79% helium and 21% oxygen. Helium has a greater thermal conductivity than nitrogen. Thus, placing animals in a He-O_2 atmosphere rather than air increases their rate of heat loss at any given ambient temperature and correspondingly elicits an elevated rate of heat production. Advantages of using an He-O_2 atmosphere to elicit peak oxygen consumption are as follows (Rosenmann and Morrison, 1974): (1) Peak oxygen consumption occurs at a much higher ambient temperature in He-O_2 than in air. Thus, refrigeration equipment capable of producing extremely low temperatures is not needed. (2) Peak oxygen consumption is elicited quickly (e.g., within 10-15 min). Thus, exposure to the cold stress need not be protracted. (3) Because the ambient temperature required to elicit peak oxygen consumption is relatively high in He-O_2 by comparison to air and because the length of exposure is short, chances of frostbite are minimized. Peak oxygen consumption may, in fact, be elicited at temperatures above freezing, thereby obviating risks of frostbite altogether.

The animal to be tested will be placed in a metabolic chamber constructed from a new 1-gal or 0.5-gal paint can (depending on animal size). The lid of the can will be outfitted with inflow and outflow ports for air, and the interior walls will be painted with Nextel Black Velvet coating (3M Co.) so as to have a high

emissivity at infrared wavelengths. The animal will rest on a platform of hardware cloth so that urine and feces will drop away. To begin with, air will be forced through the metabolism chamber at a measured rate. Later, He-O_2 will be supplied. Both the air and the He-O_2 will be supplied from compressed-gas cylinders. Rates of flow will be measured with valved Brooks rotameters and adjusted to levels necessary to keep the oxygen concentration in the metabolism chamber from falling below 18%. The rotameters will be calibrated for both air and He-O_2 using a Brooks Volumeter. The temperature inside the metabolism chamber will be controlled by placing the chamber in a thermally regulated water bath. The temperature will be set to the highest level that pretesting indicates is sufficient to elicit a maximal rate of oxygen consumption in He-O_2 (e.g., near 0 to -5°C for deer mice; Wickler, 1980). Temperature will be monitored by copper- constantan thermocouples connected to a Leeds and Northrup recording potentiometer; one thermocouple will be placed in the effluent air stream of each metabolism chamber. The oxygen concentration of gas flowing into and out of the metabolism chamber will be measured (sequentially) by passing the gas through an Applied Electrochemistry oxygen analyzer. The gas-flow system will be arranged to conform to condition B of Hill (1972a), permitting accurate calculation of the animal's rate of oxygen consumption. The animal will be permitted to adjust to the chamber and ambient temperature while exposed to air for 0.5-1.0 hr. Then the gas stream will be converted to He-O_2 for long enough periods to elicit peak oxygen consumption (ca. 15 min; Rosenmann and Morrison,

1974; Wickler, 1980). Finally, the animal will be removed and its body temperature taken promptly using a copper-constantan thermocouple inserted rectally.

In certain animals in which peak metabolic rates have been measured, the coefficient of variation of maximal oxygen consumption has proved to be 6-12% (Didow, 1972; Wickler, 1980). Assuming a coefficient of 10%, a 10% difference in peak oxygen consumption between the test and control plots could be detected at the 5% level of statistical significance with 90% surity if 22 animals from each plot were measured (Sokal and Rohlf, 1969). Because the peak rate of oxygen consumption is likely to be affected by the degree of cold stress an animal has experienced in recent weeks, and thus by weather (Lynch, 1973; Wickler, 1981), a paired experimental design will be used for comparison of individuals. That is, for each individual captured on the test plot, an individual of the same species will be collected simultaneously ($\pm$ 2 days) on the control plot. These individuals will than be paired in the statistical analysis. In total, at least 22 individuals of each species (deer mouse, squirrel, and chickadee) will be collected from each plot during December-March of each year.

Body fat content: To avoid decimating the adult populations of our plots, body fat content will be measured using a method that does not require killing animals, as decribed shortly. The animals to be tested will be captured on plots T2 and C2 using the same basic techniques as described earlier in the section on measuring peak rates of heat production. Our interest here will be in

animals nearing the end of their active phase of the day, when fat reserves can be expected to be maximal (e.g. Chaplin, 1974). Thus, chickadees, and squirrels will be captured in late afternoon, and deer mice will be trapped in the hours preceding dawn. To minimize metabolic fat depletion after capture, traps for mice and squirrels will be checked very frequently (every 0.5-1.0 hr). Mist nets will be tended continuously. Captured animals will be taken promptly to the laboratory and their fat content measured immediately. Then they will be kept in the laboratory until the start of their next active phase (dawn for chickadees and squirrels; dusk for mice), when they will be released at their point of capture. They will be fed to satiation prior to release.

The apparatus for measuring fat content is that described by Kodama and Pace (1963). The animal is anesthetized with Nembutal and placed in a sealed chamber of known volume filled with air at atmospheric pressure. Then the animal chamber is connected (by turning a valve) with a sealed, partially evacuated chamber, also of known volume. Once pressure equilibrium is attained throughout both chambers (about 15 sec after turning the valve), the equilibrium pressure is measured using a mercury manometer. This equilibrium pressure depends on the amount of air in the system. In turn, the amount of air depends on the body volume of the animal (for the animal displaced air from the chamber that was initially filled with air at atmospheric pressure). Thus, the animal's body volume can be calculated from the equilibrium pressure. By weighing the animal, its whole-body density (weight/volume) can be calculated. This density is strongly correlated with body fat

content; lean animals have relatively high whole-body densities, and fat ones have relatively low densities. Accordingly, the fat content of an individual can be calculated by knowing its density and referring to the relationship between body fat content and density. The procedure involves exposing the animal to a brief (ca. 15 sec) decompression, but such decompression does not exert adverse effects (Kodama and Pace,1963). The procedure also demands anesthetization, but recovery will be complete by the time the animal can appropriately be released (at the start of its next active phase).

In chickadees, the coefficient of variation in the percentage body fat content is about 18% (Chaplin, 1974). In mice, on the other hand, this coefficient is about 33% (Lynch, 1973; Millar, 1981). These figures are based on chemical analysis of body fat. However, coefficients of variation for determinations of body fat by measurement of body density are likely to be quite similar, based on the data of Kodama (1971). To detect a 20% difference in percentage of body fat between test and control plots at the 5% level of statistical significance with 90% surity, about 18 chickadees and 60 mice will need to be measured on each plot (Sokal and Rohlf, 1969). It may thus be possible to carry out a complete comparison of the test and control plots for chickadees each winter, but collection of adequate data on mice will likely require two winters. We have not found data on the variance in body-fat content of tree or flying squirrels, but the work on squirrels would seem likely to fall within similar boundaries to that on chickadees and mice. As in the studies of peak metabolic

rate, the experimental design will be paired with respect to individuals; thus, for each individual captured on the test plot, an individual of the same species will be collected simultaneously ($\pm$ 2 days) on the control plot.

The apparatus for measuring body-fat content will need to be calibrated for application to each species of interest. Calibration involves: (1) selection of optimal sizes for the animal and evacuation chambers (Kodama and Pace, 1963), and (2) determination of the density of fat and the density of the dry fat-free carcass (Kodama, 1971). The calibration procedures and construction of the apparatus will be carried out during the first winter of the grant period, making a fully functional apparatus available for all subsequent winters. Animals of each species will need to be killed for the calibration steps. Subdivision of their carcasses into fat and fat-free components will be carried out by ether extraction in a Soxhlet apparatus (Joslyn, 1950).

Section 5. Work Plan

Section 4, Actual Experimental Studies, describes a number of specific experiments to be carried out during the proposed research period. These experiments can be classified as studies involving: (1) Population surveys (Section 4.A), (2) Homing and Telemetry (Section 4.B), (3) Parental and Nestling Behavior (Sections 4.C and 4.D), (4) Growth, Maturation, and Fecundity (Sections 4.E and 4.F), (5) Development (Section 4.G), and (6) Thermoregulation (Section 4.H). In order to organize our efforts in executing these experiments, we have developed a work plan for each of the six experimental categories. The schedule of activities for these work plans are found on the next six pages.

SCHEDULE OF ACTIVITIES FOR POPULATION-SURVEY STUDIES

7/82	8/82	9/82	10/82
------select and survey plots-------->			
	-collect preliminary trapping data on->		
	-small mammal populations------------->		

11/82	12/82	1/83	2/83	3/83	4/83	5/83	6/83	7/83	8/83	9/83	10/83
						.----carry out trapping survey of small-->					
						.----mammal populations on study plots--->					
						.---conduct-->.					
						.---avian---->.					
						.---census--->.					

11/83	12/83	1/84	2/84	3/84	4/84	5/84	6/84	7/84	8/84	9/84	10/84
------data reduction and analysis------>.											
						.----carry out trapping survey of small-->					
						.----mammal populations on study plots---(					
						.---conduct-->.					
						.---avian---->.					
						.---census--->.					

11/84	12/84	1/85	2/85	3/85	4/85	5/85	6/85	7/85	8/85	9/85	10/85
------data reduction and analysis------>.											
						.---carry out trapping survey of small-->					
						.----mammal populations on study plots--->					
						.---conduct-->.					
						.---avian---->.					
						.---census--->.					

11/85	12/85	1/86	2/86	3/86	4/86	5/86	6/86	7/86	8/86	9/86	10/86
------data reduction and analysis------>.											
						.----carry out trapping survey of small-->					
						.----mammal populations on study plots--->.					
						.---conduct-->.					
						.--avian---->.					
						.---census--->.					

SCHEDULE OF ACTIVITIES FOR HOMING AND TELEMETRY STUDIES

	7/82		8/82		9/82		10/82
					.---test telemetry-->.		
					.--equipment at---->.		
					.--Clam Lake------>.		

11/82	12/82	1/83	2/83	3/83	4/83	5/83	6/83	7/83	8/83	9/83	10/83
							.-perform field---->.				
							.-space-utilization->.				
							.-and homing exps--->.				

11/83	12/83	1/84	2/84	3/84	4/84	5/84	6/84	7/84	8/84	9/84	10/84
------data reduction and analysis------>.											
							.-perform field---->.				
							.-space-utilization->.				
							.-and homing exps--->.				

11/84	12/84	1/85	2/85	3/85	4/85	5/85	6/85	7/85	8/85	9/85	10/85
------data reduction and analysis------>.											
							.-perform field---->.				
							.-space-utilization->.				
							.-and homing exps--->.				

11/85	12/85	1/86	2/86	3/86	4/86	5/86	6/86	7/86	8/86	9/86	10/86
------data reduction and analysis------>.											
							.-perform field---->.				
							.-space-utilization->.				
							.-and homing exps--->.				

SCHEDULE OF ACTIVITIES FOR PARENTAL AND NESTLING BEHAVIOR STUDIES

```
| 7/82-——          8/82              9/82            10/82 |
|------manufacture nest boxes----------->.                  |
|                        .                .——————install nest boxes on plots----->|
|                        .                .                                        |
|-----------------------------acquire nest monitoring equipment------------------>|
```

```
|11/82  12/82   1/83    2/83    3/83    4/83    5/83   6/33   7/83   8/83   9/83  10/83|
|----test and debug------->.                                                           |
|---monitoring equipment-->.                                                           |
|                          .   .-install &-->.                                         |
|                          .   .-test in--->.                                          |
|                          .   .-field----->.                                          |
|                          .                 .------collect parental and nestling------>|
|                          .                 .------behavioral data on small mammals--->|
|                          .                 .------and birds in the field------------->|
```

```
|11/83  12/83   1/84    2/84    3/84    4/84    5/84   6/84   7/84   8/84   9/84  10/84|
|------data reduction and analysis------->.                                            |
|                                          .------collect parental and nestling------>|
|                                          .------behavioral data on small mammals--->|
|                                          .------and birds in the field------------->|
```

```
|11/84  12/84   1/85    2/85    3/85    4/85    5/85   6/85   7/85   8/85   9/85  10/85|
|------data reduction and analysis------->.                                            |
|                                          .------collect parental and nestling------>|
|                                          .------behavioral data on small mammals--->|
|                                          .------and birds in the field------------->|
```

```
|11/85  12/85   1/86    2/86    3/86    4/86    5/86   6/86   7/86   8/86   9/86  10/86|
|------data reduction and analysis------->.                                            |
|                                          .------collect parental and nestling------>|
|                                          .------behavioral data on small mammals--->|
|                                          .------and birds in the field------------->|
```

SCHEDULE OF ACTIVITIES FOR GROWTH, MATURATION, AND FECUNDITY STUDIES

7/82	8/82	9/82	10/82
------manufacture nest boxes---------->.			
		.-------install nest boxes on plots----->	
-----------------------------acquire nest monitoring equipment-------------------->			

11/82	12/82	1/83	2/83	3/83	4/83	5/83	6/83	7/83	8/83	9/83	10/83
----test and debug-------->.											
----monitoring equipment-->.											
			.-install &-->.							
			.-test in---->.							
			.-field----->.							
					.-----collect growth, maturation, and---->					
					.-----fecundity data on small mammals---->					
					.-----and birds in the field----------->					

11/83	12/83	1/84	2/84	3/84	4/84	5/84	6/84	7/84	8/84	9/84	10/84
------data reduction and analysis------>.											
					.-----collect growth, maturation, and---->					
					.-----fecundity data on small mammals---->					
					.-----and birds in the field----------->					

11/84	12/84	1/85	2/85	3/85	4/85	5/85	6/85	7/85	8/85	9/85	10/85
------data reduction and analysis------>.											
					.-----collect growth, maturation, and---->					
					.-----fecundity data on small mammals---->					
					.-----and birds in the field----------->					

11/85	12/85	1/86	2/86	3/86	4/86	5/86	6/86	7/86	8/86	9/86	10/86
------data reduction and analysis------>.											
					.-----collect growth, maturation, and---->					
					.-----fecundity data on small mammals---->					
					.-----and birds in the field----------->					

SCHEDULE OF ACTIVITIES FOR DEVELOPMENTAL STUDIES

```
|          7/82                    8/82                   9/82                    10/82        |
|                         .                    .                      .                       |
| ---contruct thermal eggs and acquire---->.                          .                       |
| ---and test recording equipment--------->.                          .                       |
|                         .                    .                      .                       |
|                         .                    .       .------select study plots and install---->|
|                         .                    .       .------nest boxes on plots--------------->|
|                         .                    .                      .                       |

| 11/82  12/82   1/83   2/83   3/83   4/83   5/83   6/83   7/83   8/83   9/83   10/83 |
|        .       .       .       .       .       .       .       .       .       .     |
|        .       .       .       .       .    .---monitor nests,-->.     .       .     |
|        .       .       .       .       .    .---collect eggs, &->.     .       .     |
|        .       .       .       .       .    .---fix embryos---->.      .       .     |
|        .       .       .       .       .       .       .       .       .       .     |
|        .       .       .       .       .       .       .       .   .--data reduction--->|
|        .       .       .       .       .       .       .       .   .--and analysis----->|
|        .       .       .       .       .       .       .       .       .       .     |

| 11/83  12/83   1/84   2/84   3/84   4/84   5/84   6/84   7/84   8/84   9/84   10/84 |
|        .       .       .       .       .       .       .       .       .       .     |
| --data reduction--->.          .       .       .       .       .       .       .     |
| --and analysis----->.          .       .       .       .       .       .       .     |
|        .       .       .       .       .    .---monitor nests,-->.     .       .     |
|        .       .       .       .       .    .---collect eggs, &->.     .       .     |
|        .       .       .       .       .    .---fix embryos---->.      .       .     |
|        .       .       .       .       .       .       .       .       .       .     |
|        .       .       .       .       .       .       .       .   .--data reduction--->|
|        .       .       .       .       .       .       .       .   .--and analysis----->|
|        .       .       .       .       .       .       .       .       .       .     |

| 11/84  12/84   1/85   2/85   3/85   4/85   5/85   6/85   7/85   8/85   9/85   10/85 |
|        .       .       .       .       .       .       .       .       .       .     |
| --data reduction--->.          .       .       .       .       .       .       .     |
| --and analysis----->.          .       .       .       .       .       .       .     |
|        .       .       .       .       .    .---monitor nests,-->.     .       .     |
|        .       .       .       .       .    .---collect eggs, &->.     .       .     |
|        .       .       .       .       .    .---fix embryos---->.      .       .     |
|        .       .       .       .       .       .       .       .       .       .     |
|        .       .       .       .       .       .       .       .   .--data reduction--->|
|        .       .       .       .       .       .       .       .   .--and analysis---->|
|        .       .       .       .       .       .       .       .       .       .     |

| 11/85  12/85   1/86   2/86   3/86   4/86   5/86   6/86   7/86   8/86   9/86   10/86 |
|        .       .       .       .       .       .       .       .       .       .     |
| --data reduction--->.          .       .       .       .       .       .       .     |
| --and analysis----->.          .       .       .       .       .       .       .     |
|        .       .       .       .       .    .---monitor nests,-->.     .       .     |
|        .       .       .       .       .    .---collect eggs, &->.     .       .     |
|        .       .       .       .       .    .---fix embryos---->.      .       .     |
|        .       .       .       .       .       .       .       .       .       .     |
|        .       .       .       .       .       .       .       .   .--data reduction--(|
|        .       .       .       .       .       .       .       .   .--and analysis---->|
|        .       .       .       .       .       .       .       .       .       .     |
```

SCHEDULE OF ACTIVITIES FOR PHYSIOLOGICAL STUDIES

```
| 7/82 | 8/82 | 9/82 | 10/82 |
| ---------acquire and test equipment needed for physiological measurements---------> |
```

```
| 11/82 | 12/82 | 1/83 | 2/83 | 3/83 | 4/83 | 5/83 | 6/83 | 7/83 | 8/83 | 9/83 | 10/83 |
--------construct, debug, and calibrate apparatus for-------->.
--------body-fat determinations---------------------------------->.

--------determine densities of body components of species--->.
--------to be studied------------------------------------------->.
                                      .----install all equipment->.
                                      .----on site and debug----->.
```

```
| 11/83 | 12/83 | 1/84 | 2/84 | 3/84 | 4/84 | 5/84 | 6/84 | 7/84 | 8/84 | 9/84 | 10/84 |
--take measurements on winter---->.
--animals------------------------->.
                                 .---data reduction and analysis--->.
```

```
| 11/84 | 12/84 | 1/85 | 2/85 | 3/85 | 4/85 | 5/85 | 6/85 | 7/85 | 8/85 | 9/85 | 10/85 |
--take measurements on winter---->.
--animals------------------------->.
                                 .---data reduction and analysis--->.
```

```
| 11/85 | 12/85 | 1/86 | 2/86 | 3/86 | 4/86 | 5/86 | 6/86 | 7/86 | 8/86 | 9/85 | 10/56 |
--take measurements on winter---->.
--animals------------------------->.
                                 .---data reduction and analysis--->.
```

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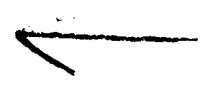Island Tree Swallows. Bird-Banding, 25: 35-58, 102-110,
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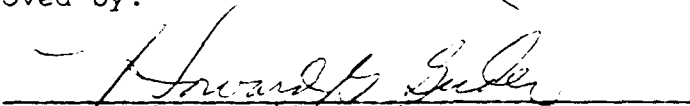
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A.   Abstract

Monitoring of the effects of ELF on stream ecosystems was
initiated in July, 1982. This report covers the first four months
of this effort. The primary emphasis during this four month period
was to selecte streams for research, obtain background dta on the
biota, and establish and test research procedures. Potential
stream research sites selected included two sites on the Ford River
in Dickinson County just west of Ralph, Michigan, two sites on the
West Branch of the Escanaba River north of Ralph, Dickinson County,
Michigan, and one site each on Schwartz and McGregor creeks,
tributaries to the West Branch of the Escanaba. Preliminary
sampling on all these streams included water chemistry as well as
periphyton samples for species identification, cell counts,
chlorophyll a/phaeophytin a production, and biomass production.
Basket samplers for sampling benthic invertebrates were placed in
the Ford and Escanaba rivers and removed at monthly intervals for
species identification, individual counts, and diversity and
secondary production estimates. Leaf packs of green and dry
speckled (tag) alder leaves were placed in the Ford River, Escanaba
River, and Schwartz Creek and will be removed at routine intervals
until decomposition is complete. Aquatic invertebrates associated
with leaf packs will be identified to species and enumerated.
Population and migration studies of crayfish populations were
initiated on the Ford River.

Fish studies included studies of population density of the long-nosed dace and sculpin on the Ford River. Food habits, fecundity, and life history studies of the long-nosed dace, sculpin, and brook trout were initiated. Parasites of fish in the study streams were identified from several species of fish. Age and growth studies of fish were begun. Correlation studies of fish feeding habits and invertebrate drift were initiated by 24-hr sampling of fish for stomach analyses and invertebrate drift for the Ford River, Escanaba River, and McGregor Creek.

Many samples from the above activities were preserved for laboratory analyses during winter months. Thus, no conclusions are possible at this time.

B.   Summary:

Initial efforts were directed towards identifying control and experimental sites on the two major river systems that cross the proposed corridors.   These two are the Ford River and the West Branch of the Escanaba.   Both sites are prime brook trout habitats and are considered valuable resources by the local fishermen.   Two second order tributaries of the Escanaba, McGregor Creek and Schwartz Creek, were also sampled.

The following is a summary of the data based collected from 10 July to 31 October, 1983, summarized by work plan element:

Element 1 - **Paired Plot Selection**

Stream Order 4:   Two sites on the Ford River (R29W,T43N)

Stream Order 5:   Two sites on the West Branch of the Escanaba River (T44N,R27W)

Stream Order 2:   One site on Schwartz Creek (T44NR28W, Sect. 11)

One site on McGregor Creek (T44N,R28W, Sect. 25)

Element 2 - **Inventory Physical Characteristics of Stream Sites**

24 benthic substrate samples (3 replicates in pool and 3 replicates in riffles) from all sites; seived into 7 size categories. Velocities, widths and depths taken at the stream order 3 and 4 sites. Riparian vegetation characterized for all except for McGregor Creek site.

Element 3 - <u>Establish and Conduct Ambient Monitoring Program</u>

Continuous monitoring stations selected for installation in the spring of 1983. Chemical background sampling at all sites, with intensive sampling at the 4th order stream--the Ford (3.2 samples per week from August 1 - November 1, 1982.) Twenty-four hour chemistries taken on the 3rd and 4th order stream sites (including D.O., pH, specific conductance, turbidity, alkalinity and hardness).

Element 4 - <u>Effects of Exposure Period on Periphytic Colonization of</u> <u>Artificial Substrates</u>

Periphyton slides collected on days 4, 7 and 14 and then every 7 days thereafter for 8 weeks from the two sites on the 4th order stream and the two sites on the 3rd order stream. Initial stages of colonization studied in the 4th order stream by collecting slides on days 4, 5, 6, 9, 10 and 11. Biomass accumulation and chlorophyll <u>a</u>/phaeophytin <u>a</u> production over time determined on the 3rd and 4th order streams.

Element 5 - <u>Compare Periphyton Communities on Natural Substrates with</u> <u>those on Artificial Substrates</u>

Periphyton sampled from rocks, sediment, and wood at all study locations for later comparative purposes.

Element 6 - <u>Monitoring of Species Composition, Numbers, Diversity,</u>

              <u>Biomass</u>

              Determination of the best exposure interval (12 days)
for chlorophyll a/phaeophytin a biomass accumulation.
Studies were done in sites from all stream orders.

Element 7 - <u>Stream Invertebrate Collection and Identification</u>

              Establishment of a macroinvertebrate species list, based
on samples taken from all stream orders.

Element 8 - <u>Effects of Exposure Period on Invertebrate Colonization of</u>

              <u>Artificial Substrates</u>

              Artificial substrates sampled after 4, 7 and 14 days in
pools and riffles from the 3rd and 4th order streams.

Element 9 - <u>Species Richness and Biomass of Stream Macroinvertebartes</u>

              <u>in Riffles and Pools from Artificial Substrates</u>

              Five replicates from the 3rd and 4th order streams
collected after 21 and 54 days for a total of 20
samples.

Element 10 - <u>Movement Patterns of Selected Aquatic Invertebrates</u>

              Population estimates for the crayfish <u>Orconectes</u> sp.
were determined for the 4th order stream. Individuals
were marked with non-toxic enamel and directions moved
were recorded.

Element 11 – <u>Colonization Patterns and Processing by Invertebrates on</u>
<u>Autumnal Freshly Fallen Leaves</u>

Speckled (Tag) Alder leaves put in one 2nd order stream
(Schwartz Creek) and the 3rd and 4th order streams.
Five replicates from each stream collected after 3, 9
and 27 days (45 samples). White Birch leaves treated
the same way but placed only in the 4th order stream.
Freshly picked Speckled Alder leaves put in the 3rd and
4th order streams, with 3 replicates taken after 3, 6,
12, 25 and 54 days. This was repeated for the 3rd and
4th order streams and one 2nd order stream (Schwartz
Creek), with 5 replicates being collected after 3, 9,
27 and 54 days.

Element 12 – <u>Drift Patterns of Aquatic Invertebrates</u>

24-hr drift studies completed on the 3rd order stream
at two-hr intervals (72 samples), and on the 4th order
stream at two- and then three-hr intervals (54
samples).

Element 13 – <u>Leaf Litter Processing Experiments Using Natural Leaf</u>
<u>Packs and Cages</u>

Preparatory to this study, litter collecting traps were
set above water in the 3rd and 4th order streams and in
Schwartz Creek. Samples were collected and assayed 10
times. Also, amoung and type of leaves in drift
(Element 12) were assayed. Caged experiment will be

done in the fall of 1983.

Element 14 - <u>Feeding Activity of Grazer Populations</u>

Major grazing taxa identified in the 3rd and 4th oder streams. Qualitative gut content analysis on <u>Leucotrichia</u>, a caddisfly were initiated.

Element 15 - <u>Fish Species Composition, Relative Abundance and Habitat Relationships</u>

Fish surveys were done in the 3rd and 4th order streams. 14 species were collected.

Element 16 - <u>Assessment of Equipment Efficiency for Capture of Selected Fish Species</u>

Net, kick seines, minnow traps and directional fyke nets were tested. The latter proved to be most effective; however, new methods of collection are currently under evaluation.

Element 17 - <u>Age-Length-Weight Relationships; Growth, Fecundity, Survival, and Distribution of Selected Fish Species</u>

Vital statistics are currently being determined for collected fish populations.

Element 18 - <u>Diurnal Food Habits and Consumption Rates of Selected Fish Species</u>

Food habits of brook trout, sculpins and longnose dace from 3rd and 4th order streams were examined.

Element 19 - <u>Mark-Recapture Studies of Sculpin</u>

Initial population estimates were determined for the longnose dace and sculpin in the 4th order streams.

Element 20 - <u>Studies of Patterns of Development from Egg to Adult for Selected Fish Species</u>

Brook trout development patterns will be studied during spawning in October of 1983.

Element 21 - <u>Parasite Loads of Selected Fish Species</u>

Individuals of 14 fish species have been examined (both alive on site and preserved specimens) for parasite loads. 15 genera of parasites have been identified from eyes, gills flesh, swim bladder, viscera and digestive tracts.

Element 22 - <u>Data Analysis and Report Writing</u>

Data management forms and statistical packages have been designed for our data collection, tabulation and analyses.

PROJECT RATIONALE AND APPROACH

The potential impact of any environmental perturbation on an ecological community can be assessed at several levels of organization ranging from individual responses to community and ecosystem responses. At times, even a comprehensive understanding of individual species biology will not predict the complex interactions, interlocking cause and effect pathways, and operational characteristics of communities (Cairns et al. 1972; Cairns 1981). In essence, our current state of understanding of the biology of individual species populations is not sufficient to allow prediction of community or ecosystem effects. Thus, any assessment of potential environmental damage from extremely low frequency electromagnetic fields has to include assessment of individual and population responses, but more importantly should include community and ecosystem responses as well.

The appropriate community or ecosystem parameters for such environmental assessment may vary from community to community. In our experience, process or functional types of parameters are often the most sensitive and least variable integrated indicators of environmental stress. For example, studies conducted by T.M. Burton on the effects of acid rain on stream invetebrate organisms in Michigan demonstrated that organic matter processing rates of experimental leaf packs and the density, composition, and survival of the invertebrate leaf pack community was an excellent method of quantifying this stress (Burton et al. 1982). Likewise, studies by J. Stout and others on the effects of organic toxicants

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on stream ecosystems at Monticello, Minnesota, also suggested the utility of this approach as well as the utility of pre-placed substrates of natural bottom material of known area for sampling the effects of stress on the benthic community (Cooper and Stout 1981; Stout and Cooper, In Press).

A recent EPA sponsored study corroborated our experience that many ecosystem properties are sensitive to toxicant perturbations (Hendrix et al. 1981). This study suggested that community metabolism was a very sensitive indicator of stress, especially net daytime production and nighttime respiration. They also found that densities of various taxonomic groups provided a consistent indicator of toxicant stress, expecially community composition by trophic groups. Another sensitive indicator for their study of cadmium stress was output-input ratios of NO_3-N, Mn, and Fe. This latter indicator may have been related to selective effects of cadmium on nitrogen metabolism and thus might not be a useful indicator for other types of stress.

Certain life history stages are more subject to damage than are others, with embryonic and juvenile stages often being highly sensitive to environmental stress. Because the effects of ELF may be subtle, initial damage to these stages may be in the form of behavioral effects rather than in mortality schedule effects. Since most aquatic vertebrate and invertebrate species are mobile, both potential effects can be monitored. Such individual life history data are an important part of a complete study of stress on the community.

Thus, in our original research plan, we proposed an integrated

study of stream ecosystems involving three aquatic components for monitoring the potential effects of ELF. These components were: 1) periphytic algae; 2) aquatic insects; and 3) fish. The design incorporated studies of ecosystem properties with studies of behavior and biology of individual species so that any effects of ELF would be quantified at the population, community and ecosystem levels.

We selected stream ecosystems as representative aquatic ecosystems rather than lakes or marshes because: (1) upstream-downstream paired plots on the same system would provide less variability than between lake comparisons; (2) migratory behavior was more likely to be important in stream organisms; and (3) our local expertise and interests were oriented more toward stream ecology.

We planned to test the effects of ELF on stream ecosystems by using a paired "plots" design on selected sections of a chosen stream. Specific control and experimental sites were to be selected after the final ELF cable corridors were established. We planned to select a stream section containing pools and riffles in an area of forest just upstream of the cable corridor with maximum exposure to extremely low frequency electromagnetic radiation (ELF). This section was to be compared to a physically similar site (with regard to depth, width, flow rates, canopy cover, etc.) on the same stream far enough away from the ELF cable to receive at least an order of magnitude less exposure to ELF. The two stream sections constituted our paired "plot" design. Thus we planned to have two plots of intensive stream studies: a control site and

an experimental site at the cable corridor. We expected these studies to continue for a least 3 years of preconstruction background data collection followed by at least 2-3 years of post construction data collection.

For each site, we planned to continuously monitor stream velocity and water depth so the discharge could be calculated. Water and air temperatures, dissolved oxygen, pH, solar radiation at the water surface and at the stream bottom, and relative humidity were also be continuously monitored. We planned to sample all other required chemical parameters required in the RFP as detailed in the work plan submitted in 1982/83 (Element III of this report).

In conclusion, the proposed research is directed at determining the effects of low-level, long term electromagnetic fields and gradients produced by the ELF Communications System on aquatic plant and animal life. The integrated approach we have taken is to combine the major interrelated and interactive components of aquatic systems (i.e., periphytic algae, aquatic insects, and fish) and to monitor sensitive life history events and community processes critical to the basic structure and function of stream ecosystems. These include: periphyton and stream inverte- brate colonization, migration, diversity, trophic level changes in density and biomass, as well as primary productivity; organic matter processing by macroinvertebrates; dynamics of fish population growth, reproduction, and survival; fish behavior including movement patterns of homing and migration, and fish pathogen and parasite loads. Since many of these processes and

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events are mutually dependent on one another and the interactions

are complex, we feel that a holistic approach with a

multi-disciplinary effort is imperative.

The data generated from the proposed research should:

(1) determine whether the ELF Communications System affects aquatic

plant and animal life in stream systems; and (2) contribute to a

better understanding of stream organism processes which will help

clarify a number of important aspects of current conceptual models

of stream ecosystem structure and function.

OVERALL OBJECTIVES AND SPECIFIC TASK OBJECTIVES

OVERALL OBJECTIVE

Our major objective in this study is to determine the effects of low level, long term electromagnetic fields and gradients produced by the ELF Communication System on aquatic plant and animal life in streams. The study will incorporate studies of ecosystem properties with studies of behavior and biology of individual species so that any effects of ELF will be quantified at the population, community and ecosystem level.

SPECIFIC TASK OBJECTIVES

A. **Periphytic Algal Studies**

The objectives of the periphytic algal studies are:

(1) to quantify any changes in species diversity, algal density, and chlorophyll $\underline{a}$ that occur as a result of ELF electromagnetic fields;

(2) to quantify any changes in primary productivity that might occur as a result of ELF; and

(3) to monitor algal cell volume and chlorophyll $\underline{a}$ to phaeophytin $\underline{a}$ ratio, thereby providing an index to physiological stress of periphytic algal cells that might occur as a result of ELF electromagnetic fields.

B.   Aquatic Invertebrate Studies

The objectives of the studies of aquatic invertebrates are:

(1)   to quantify any changes in organic matter processing rates that occur as a result of ELF;

(2)   to quantify changes in species richness, individual abundances, and species diversity of the aquatic invertebrate communities associated with leaf packs and inorganic stream bottom substrates;

(3)   to quantify changes in upstream-downstream movements of selected aquatic invertebrates that might occur as a result of ELF; and

(4)   to quantify trophic, behavioral, and community level changes in selected species of aquatic insect "grazers" or "scrapers" which feed on algae or periphyton attached to stream substrates.

C.   Fish Studies

The objectives of the study are to determine the effects of electromagnetic radiation using 2 to 3 species of fish on:

(1)   feeding, growth and production;

(2)   fecundity;

(3)   rates of parasitism;

(4)   migration;

(5)   behavioral habitat selection; and

(6)   recruitment and survivorship.

The above studies and objectives will be coordinated and
integrated with the ambient monitoring program.

PROPOSED RESEARCH

A. Periphytic Algae

    1. Introduction and Objectives:

The diversity of the periphytic community and shifts in
this diversity have been suggested as being sensitive indicators of
environmental stress (Patrick, 1973; Cairns et al., 1972). Much of
the early work in this field was conducted by Patrick and
co-workers (reviews by Cairns, 1977; Cairns et al., 1972; Patrick,
1973) with special emphasis on the diatom community.

Variations of Patrick's "diatometer" technique are used
routinely for monitoring effects of environmental stress, and
similar techniques have been used to quantify the role of the
protozoan community in aquatic systems under stress (Cairns et al.,
1972; Cairns and Henebry, 1982). A variety of artificial
substrates have been used for these types of studies but the most
commonly used substrates are: (1) glass slides, (2) plastic petri
dishes and plastic cover slips, (3) polyurethane foam (PF), and (4)
nylon reticular slides and grids (Bamforth, 1982). Many of the
studies conducted in Michigan streams have used plexiglass slides
rather than glass (e.g., Burton and King, 1982; Grzenda and
Brehmer, 1960; King and Ball, 1966, 1967).

Bamforth (1982) compared the polyurethane foam, glass slide,
and plastic petri dish techniques and found that each had
advantages and disadvantages but that all three showed similar

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trends in numbers of individuals and numbers of species as
environmental conditions changed. Thus, selection of a specific
technique is primarily one of investigator preference, and we have
chosen the glass slide technique.

Glass slide techniques can also be used for determination of
biomass accumulation and productivity of periphyton and chlorophyll
a (Sladecek and Sladeckova, 1964; King and Ball, 1966). Since
productivity and biomass accumulation can indicate changes in
environmental stress (Burton and King, 1982), this technique
should prove useful in determining any impact of ELF on the aquatic
ecosystem.

All artificial substrates are biased since they accumulate
only a portion of the community (Bamforth, 1982). Since community
respiration and productivity are known to be sensitive indicators
of environmental stress (Hendrix et al., 1981), we propose to
expand the periphytic algal studies to include these parameters. We
will use pre-placed natural substrates in open trays for long-term
colonization prior to employing metabolic studies in standard
metabolism chambers (Bott et al., 1978).

2. Experimental Design:

a. Periphyton Biomass:

The periphytic community at each of the two stream
sites will be monitored using the glass slide technique as
described by Bamforth (1982). Glass slides will be placed in a
specially built plexiglass "slide box" designed to hold 6 pairs of
glass slides. Four slide boxes will be placed just above the
sediment at randomly selected locations in the riffle area and from

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boxes will be placed in the pool area of the study section. The 6 slides will be removed from slide boxes after routine intervals of 7-28 days and replaced with 6 new slides.

Artificial substrates in running water tend to establish an equilibrium numbers of species after 3 to 7 days of immersion (Cairns and Henebry, 1982). Biomass accumulation plateaus occur after 14 to 21 days in southern Michigan rivers (Burton and King, 1982; King and Ball, 1967). After biomass plateaus are reached, colonization rates equal sloughing rates. In order to quantify rate of accumulation as a measure of productivity (King and Ball, 1966), it is desirable to sample prior to the biomass accumulation plateau. Thus, an interval between 7 and 28 days is likely to be the correct exposure time. Initially, time sequenced exposures will be sampled to determine the correct exposure time; exposure time will be adjusted as necessary throughout the season.

Two of the 6 slides removed from each sampler at appropriate intervals will be transferred under water to bottles of water where they will be preserved. These slides plus the water in the bottle will be used for counts of individuals; two of the 4 remaining slides will be used for chlorophyll a, and phaeophytin a, and the remaining 2 will be used for biomass determination by determining ash-free dry weight and oven-dry dry weight, using methods outlined in Standard Methods (A.P.H.A., 1976). These data will be used to calculate productivity of the entire attached community (see methods in Burton and King, 1982; King and Ball, 1966, 1967).

Since phaeophytin a is a breakdown product of chlorophyll a (A.P.H.A., 1976), the chlorophyll a to phaeophytin a ratio will be

calculated as an indication of the physiological condition of the periphytic algae.

This sampling design will yield four samples for each determination (4 from each of the two randomly placed slide boxes), for each habitat (pool versus riffle), for each study section every 7-28 days throughout the spring, summer, and fall seasons. These samples will be processed at our field laboratory or preserved and transported back to Michigan State University for analysis during winter months.

b. Production and Respiration:

Since the glass slide technique is somewhat biased (Bamforth, 1982) and since stream metabolism is an important community level indicator of stress (Hendrix et al., 1981), we propose to conduct studies of primary productivity and community respiration using the chambers described by Bott et al. (1978). These studies will be conducted in riffle areas. The data will be used to compute an index of changes in production and respiration for the stream.

Trays of natural substrate with predetermined known surface area selected to simulate average stream substrate conditions will be placed at random locations in riffle areas of each of the two stream sites. At one month intervals, three of these trays of substrates will be placed in three standard productivity-respiration light chambers and an additional three will be placed in dark chambers for each stream site. Production-respiration measurements will be taken, using the methods of Sumner and Fisher (1979) modified to use the standard chamber design

recommended by Bott et al. (1978). These measurements will be run on successive days for thhe experimental and control stream sites. Correlation of production and respiration with photosythetically available light (PAR) will allow extrapolation of production and respiration on an annual basis.

In addition to community production and respiration measurements provided by these chamber studies, 24 hr graphical plots of dissolved oxygen from automated stream monitoring stations will enable us to plot community metabolism for each stream site, using the single point method (Wetzel and Likens, 1979). These data will constitute important daily records of community metabolism and will supplement the more detailed periodic studies already described.

In addition, diurnal and long-term trajectories of the pH-dissolved oxygen phase plane calculated from data derived from the ambient monitoring stations at each site provide a potentially sensitive measure of the state of an aquatic system and its reaction to stress (Schindler et al., 1980; Waide et al., 1980). Thus, continuous monitoring of pH and dissolved oxygen will not only provide background monitoring of water quality but may also provide a sensitive way of monitoring stress due to ELF.

The ambient monitoring program will also provide data on nutrients (e.g. N, P, Si, K, etc.) necessary for correlation of rates of production and respiration with these parameters. Site to site differences could be correlated with differences in nutrient availability, although we will attempt to select sites which

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minimize such differences. The ambient monitoring data also include

needed information on solar radiation, water temperature, stream

discharge, etc. Thus, the ambient monitoring data are an important

part of this proposal since these data are required for

interpretation of results.

B. Aquatic Insects

 1. Introduction:

   The aquatic insect studies will include all stream

invertebrates and not just insects. Two levels of approach will be

emphasized. First, community level responses will be studied,

since these responses are sensitive indicators of stress (Burton et

al. 1982; Cooper and Stout, 1981; Cairns, 1981; Hendrix et al.,

1981). These community level responses include: 1) leaf litter

processing, 2) invertebrate colonization patterns on leaf litter

and artificial substrates, and 3) the often used structural

descriptors of community change such as species richness,

individual abundances and species diversity (see Whilm and Dorris,

1966; Sanders, 1968; Tramer, 1969; Allan, 1975; Cornell et al.,

1976 for similar studies).

   The second type of approach emphasizes changes in individual

behavior such as alterations in migration or movement patterns

attributable to ELF electromagnetic fields, and changes in life

cycles, body size, growth rate, and population density for selected

representative species of aquatic invertebrates. These studies

will include representatives of the more important stream

invertebrate functional groups, such as shredders of coarse
particulate organic matter (CPOM), collectors of fine particulate
organic matter (FPOM), grazers of periphyton, and predators
(Cummins, 1978; Merritt and Cummins, 1978). Particular emphasis
will be placed on studies of the grazer food chain. These
functional groups will include chironomids, mayflies, caddisflies,
and stoneflies as specified in the RFP.

   2. Organic Matter Processing Studies

      a. General Background

         The maintenance of community structure and function
in small streams (1st-3rd order tributaries) is largely dependent
on the input of organic material from the autotrophically dominant
terrestrial system (the watershed) through which the stream flows
(Anderson and Sedell, 1979; Sedell, et al., 1975; Cummins, 1975,
1978; Cummins and Klug, 1979; Merritt and Lawson, 1979; Vannote et
al., 1980). It has been proposed that allochthonous detritus input
in first to third order woodland streams may support over 99% of
the annual energy requirements for primary consumer organisms
(Fisher and Likens, 1973). Nelson (1969) and Hynes (1975) have
inferred that the input of organic and inorganic materials to
streams arises from the valleys and landscapes that they drain, and
it is largely the events in the terrestrial environment that
determine the quantity and quality of materials streams receive.
The energy derived from the adjacent watershed enters the stream
through the following pathways: (1) as coarse particulate organic
matter (CPOM, particles generally greater than 1 mm in

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diameter) in the form of leaves, needles, twigs, branches, petals, etc., (2) as fine particulate organic matter (FPOM, particles that range in size from .45 m to 1mm) whether from upstream areas, groundwater seepage, terrestrial runoff or as invertebrate frass inputs, etc,; and (3) as dissolved organic matter (DOM, organic material smaller than .45 m) from leachate from the forest canopy or as inputs from upstream areas or groundwater seepage. After the introduction of CPOM into the stream, soluble organic matter is leached out, the majority occurring within 24 hours of the initial wetting (Petersen and Cummins, 1974). The next event is the colonization and penetration of CPOM surfaces by microorganisms, mainly bacteria and aquatic hyphomycete fungi (Suberkropp and Klug, 1976). The leached, microbially colonized CPOM is then subjected to the mechanical disruption wrought by physical abrasion in a lotic environment, microbial metabolism, and the action of animal feeding by stream deritivores.

The macroinvertebrate consumers responsible for "processing" organic material have been partitioned on the basis of their primary feeding activity into functional groups known as "shredders" (large particle ingesting detritivores such as caddisflies and crane flies), "collectors" (small or fine particle ingesting detritivores such as black flies and mayflies), "grazers" (scraping algal feeders) and predators (animal ingestors) (Merritt and Cummins, 1978). This functional group concept focuses on the mechanism of obtaining food rather than what is eaten (c.f., Cummins and Klug, 1979; Wallace and Merritt, 1980).

Any factor that retards decomposition rates is likely to result

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in reduced processing rates and less invertebrate biomass and production. In turn, fish production could be adversely affected since there is a density dependent relationship between food consumption, biomass, and growth and production of trout (Brocksen et al., 1968).

The importance of allochthonous detritus as the energy source in a stream declines as stream width increases for middle order streams (Vannote et al., 1980). Increased stream width decreases shading from the adjacent forest and increases light penetration to to the stream resulting in a switch from heterotrophy to autotrophy (Vannote et al., 1980, Naiman and Sedell, 1980). Nevertheless, some leaf fall still occurs along stream margins, and leaf litter processing studies in these middle order streams still provide a means o assessing environmental stress. In fact, leaf decomposition bioassay studies have been used to monitor effects of an organic toxicant (p-cresol) and acid rain in open channels (Cooper and Stout, 1981; U.S. EPA, 1979).

Leaf decomposition and invertebrate colonization patterns on leaves in unstressed streams have been studied extensively (Cummins et al. 1973, Iverson 1973, Kaushik and Hynes, 1971, Petersen and Cummins, 1974, Reice 1974, Short et al., 1980, Stout, 1980). Both decomposition and colonization processes concern community level responses to changing environments. Because these processes have been most often studied in unstressed systems, they are appropriate community level processes for study in stressed stream ecosystems. The studies outlined below should provide a significant procedure for the assessment of any subtle changes to the detrital community

that may result from ELF.

   b. Experimental Design:

   The methods employed will consist of field efforts
focusing on the selected stream under control and experimental
conditions. The sections will be chosen as a function of habitat type,
sediment composition, riparian vegetation, flow rate, width, and
accessability. Every effort will be made to match all relevant system
characteristics for the paired-plot (control and experimental) design.

   Studies conducted in each area will include organic matter
processing rates by various functional groups using experimental
leaf packs (Fig. 1). The leaf pack "bioassay" procedure (Merritt et
al., 1979) uses pre-weighed (dry) amounts of leaves of known
species composition and history (i.e., collected in nets from trees
after abscission). Leaves of the predominant riparian species
collected during autumn leaf fall will be air dried, and stored for
later use. The leaves, bound loosely in packs and anchored to bricks
will be placed in the stream facing into the current so as to simulate
natural accumulations of litter at the leading edge of obstructions
(Merritt et al., 1979). The actual dry weight of fresh leaves will be
determined using a regression between leaf area and lea dry weight
since we have found that initial drying changes subsequent
decomposition rates (Stout, 1981). Changes in leaf area per time will
be related to weight loss using the dry weight/leaf area ratio
technique (Stout, 1981). We will also use ash free dry wt. values by
ashing material after dry weights have been obtained. This estimated
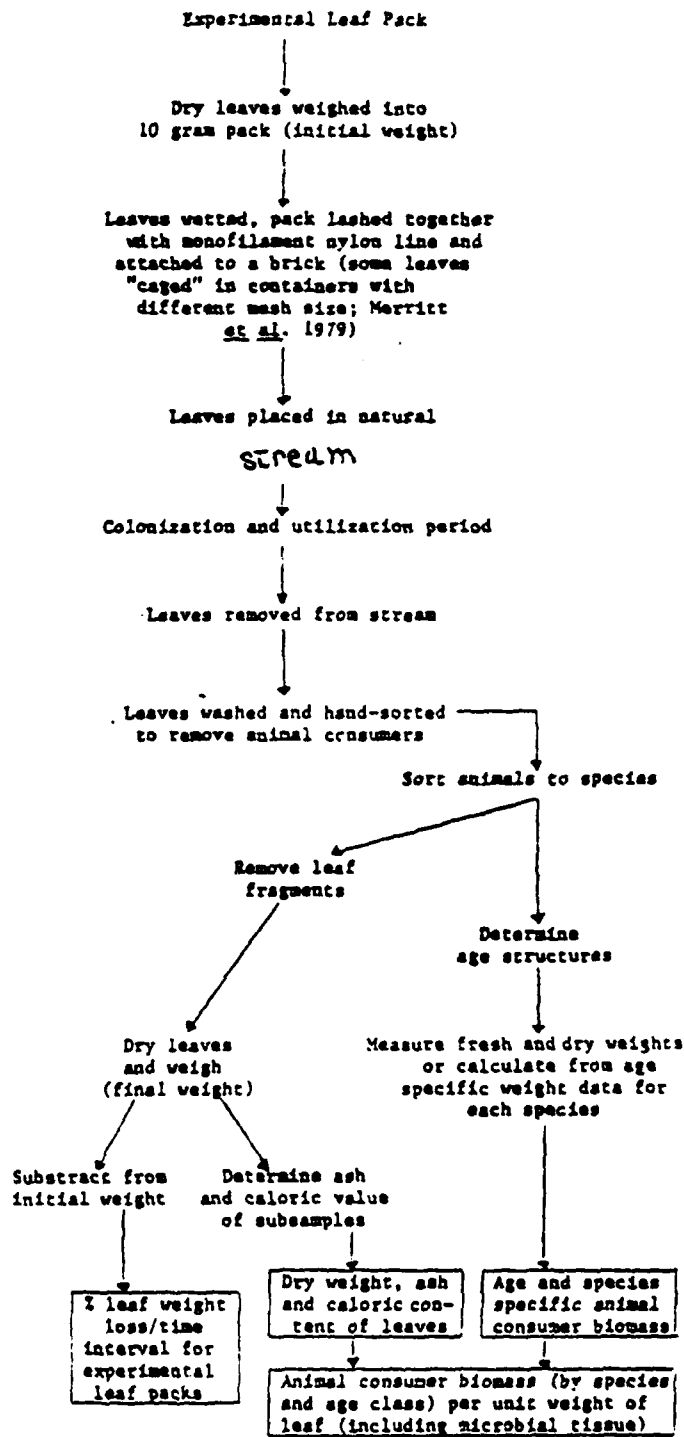weight loss will be taken as an overall estimate of CPOM detrital
processing.

Experimental Leaf Pack

↓

Dry leaves weighed into
10 gram pack (initial weight)

↓

Leaves wetted, pack lashed together
with monofilament nylon line and
attached to a brick (some leaves
"caged" in containers with
different mesh size; Merritt
et al. 1979)

↓

Leaves placed in natural

stream

↓

Colonization and utilization period

↓

Leaves removed from stream

↓

Leaves washed and hand-sorted ——→ Sort animals to species
to remove animal consumers

Remove leaf   Determine
fragments   age structures

Dry leaves   Measure fresh and dry weights
and weigh   or calculate from age
(final weight)   specific weight data for
                               each species

Subtract from   Determine ash
initial weight   and caloric value
                   of subsamples

┌──────────────┐ ┌──────────────┐ ┌──────────────┐
│ % leaf weight│ │Dry weight, ash│ │Age and species│
│ loss/time   │ │and caloric con-│ │specific animal│
│ interval for │ │tent of leaves │ │consumer biomass│
│ experimental │ └──────────────┘ └──────────────┘
│ leaf packs │ ┌──────────────────────────────────┐
└──────────────┘ │Animal consumer biomass (by species│
                  │and age class) per unit weight of │
                  │leaf (including microbial tissue)   │
                  └──────────────────────────────────┘

Fig. 1. Procedures followed in pack experiments.

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Eighty leaf packs will be placed at random locations within the riffle and pool areas of the two stream sites. Five leaf packs will be removed at 3-30 day intervals from each pool and each riffle site until decomposition is > 80% complete. The invertebrates on these leaf packs will be removed, identified, counted, measured, and weighed. The leaf packs will be dried and final dry and ash free day weight loss determined at each 14 day interval to determine processing rates. New leaf packs will be placed in the streams every autumn during litter fall for the area as bioassays of processing rates and to detect changes in leaf litter macroinvertebrate community structure.

Species richness, evenness (J'), individual abundances, and species diversity (H') of the leaf pack aquatic invertebrate community will be calculated from these data following methods of Stout and Cooper (in prep).

3. Stream Substrate Sampling:

a. General Background

Qualitative and quantitative changes in the benthic invertebrate community at specific sites in the study area will be monitored throughout the duration of the study for two major reasons: 1) to determine seasonal versus environmentally induced changes in the community, and 2) to serve as a data base for complementary studies on fish dynamics, colonization of invertebrates on leaf packs, and aquatic invertebrate movement patterns.

Monitoring will be effected through the use of artificial substrates. Artificial substrates have several advantages over destructive sampling methods); 1) they reduce variability among samples reducing the required number of replicates as compared to

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destructive sampling techniques (i.e., Surber samples or handscreen methods); 2) they allow for accurate computation of surface area sampled; 3) they are inexpensive to operate; 4) they are easier to process and quantify than other techniques; 5) they permit non-destructive, repeated sampling over time, and 6) they can be used to study either dynamic colonization rates or to estimate seasonal changes in population structure, depending on incubation periods in the stream (Flannagan and Rosenberg, 1982).

Since we will use artificial substrates to monitor changes in the invertebrate community and in food availability for fish populations, we will select incubation periods that will be long enough to approximate full colonization and short enough to detect losses of species due to seasonal biotic and abiotic events; e.g., emergence peaks, spating, etc. The incubation periods, will be determined at the site because the dynamics of colonization rates and emergence times vary among streams. Cooper and Stout (in prep) found the balance between colonization and emergence to be from 3 to 5 days for riffle and pool areas for artificial stream channels at Monticello, Minnesota during the summer periods. Colonization may be longer for more natural streams at the site (See Rosenberg and Resh, 1982, for time estimates based on other studies). Gersabeck and Merritt (1979) found peak densities of blackfly larvae on artificial substrates in some Michigan streams to occur between 5 and 7 days. We will use a monthly incubation period initially and adjust this period if necessary.

b. Experimental Design

Plastic baskets (18 cm x 29 cm) with Nytex 60 micron mesh netting

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covering the inside will be filled with natural substrate from the stream bed. Filled baskets will be submerged to so that sediment surface will be flush with surrounding substrates.

Studies of the invertebrate community will emphasize sampling throughout the year to 30 day intervals. At the beginning of the year, 60 baskets will be placed at random locations in the riffle areas of each of the two study sites. Five of these baskets will be removed at monthly intervals throughout the year. Monitoring will be initiated by late summer of 1982 and will continue until the termination of the research period.

After removal, each basket will be carefully placed in a bucket just as it breaks the water surface to minimize invertebrate loss. The buckets will be returned to the lab for processing. The substrata and mesh netting will be washed and the residue collected in 60 micron mesh seives. Collected invertebrates will be preserved for later analysis.

Analysis of the invertebrate samples will follow those presented by Crowder and Cooper, (1982) and Stout and Cooper loc. cit. where numbers of individuals, species and biomass estimates for each species will be determined. We will utilize estimates to capsule widths, post genal widths or other frequency histograms will be constructed to determine specific instars (Haefner and Wallace, 1981; Merritt et al., 1982). Production will be calculated using both the Hynes method (Hynes and Coleman, 1968) as modified by Hamilton (1969) and the removal-summation method outlined by Waters and Crawford (1973).

4. Migration of Stream Invertebrates:

There are several techniques whereby underwater movements of

invertebrates can be determined. They include: 1) underwater observations under laboratory or field conditions (Hayden and Clifford, 1974); 2) tagging individuals and then watching their movements (Hart and Resh, 1980); 3) construction of traps that capture only animals moving upstream (Hultin, 1971; Meijering, 1977; Otto, 1971; Williams, 1977); 4) dyeing groups of individuals with fluorescent pigments and then observing them with blacklights (Brusven, 1970); and 5) individually marking animals with non-toxic enamel, and recapturing and remarking animals over time (Elliott, 1971; Stout, 1978, 1982). Each technique has disadvantages, the three main being, 1) disturbance of habitat during recapture, 2) time intensive nature of underwater observations, and 3) lack of detection of all vector movements and distances travelled. We chose two techniques, which minimize disadvantages and allow quantification of movement patterns. They consist of: 1) differentially marking animals, depending on their location of capture, with fluorescent dye, and 2) individually marking animals and resampling at specified time intervals along stream widths, both upstream and downstream of release sites. The combination of these two methods will allow us to mark both fragile gill-breathing species (caddisflies, mayflies, stoneflies) and more hardy plastron and air breathing species (e.g., beetles, snails, true bugs, some dipterans). These techniques are as follows.

1. Fluorescent dye technique: Brusven (1970) has shown that fluorescent pigments (Day Glo^R) will adhere to membranes of aquatic insects. We will collect invertebrates from soft substrata with dip nets and from rocks in riffle areas and carefully transfer them to

fine mesh cages partially submerged in water into which the dye will be introduced. After the non-toxic dye has adhered to the integuments, we will release the invertebrates at the capture site. Blacklights will be used to follow general movements of the group-marked animals during the evening and night hours. At fixed intervals from the release site, locations will be selected for collection sites. We will handscreen those areas in early morning and/or evening hours and bring specimens into the laboratory for fluorescence detection. Distances and direction travelled will be recorded.

2. Individual marking techniques: Stout, (1978, 1982) has shown that a number of aquatic invertebrates (true bugs, snails, beetles) can be marked by painting a dot of colored Testors[R] enamel on the dorsum of sclerotized parts of invertebrates. We will collect appropriate invertebrates and will groupmark series (a different color for each day of the marking) of species, record numbers and colors, and then release them at the capture site. Because re-collection techniques require stirring of substrata, these experiments will be performed downstream of the previously designated experimental and control areas. Distances, directions moved, and individual numbers of each species will be recorded. Tests for homing behavior response to disturbance will be done. We will collect animals at three adjacent locations, code them as to location and then change their original sites. The same procedures will be used again except that animals will be placed in their original collection sites. Tests will be done for actively moving animals. The null hypothesis is that animals will continue to move in the same direction as before, irrespective of their relocation sites; alternatively, if they move

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back to their original locations, the data will be used to support the hypothesis that the animals are exhibiting homing behavior. These homing tests will monitor any changes in habitat selection and in stability of home ranges as a function of ELF.

Movement patterns using technique 1 will be determined for a three-day period each month. Comparisons between day and night time movement rates will be done since Elliott, (1971) showed that aquatic invertebrates tend to move more at night than during the day. General nighttime movement rates will be determined using blacklights each of the three nights of sampling. A distinction between nighttime and daytime movement for specific species will be done by handscreen sampling early in the morning and then at dusk, and taking the collected animals back to the laboratory for detection of fluorescence.

Movement patterns using technique 2 will be over a seven-day period each month. Two to three species will be selected for individual marking. Those species will be designated at the site and will be a function of density, trophic importance and mobility. After 200 to 300 individuals of each species have been collected downstream of the intensive sites, they will be marked and then released at the mid-point of the collection area. Recollections will be made 12, 24, 72, 120, and 168 hours later.

Homing behavior studies will be done separately for actively moving species. The two phases (replacement at original collection sites; replacement at reciprocal sites) will be effected over a six-day period, each phase taking three days with two recollection periods for each phase.

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C. Fish Studies

1. Introduction

Fisheries assessment and management require knowledge concerning the dynamics of fish populations (Ricker, 1977). Basic questions regarding production, reproduction, growth, food assimilation, movements, and causes and extent of mortality, must be investigated to understand and properly manage a fish population (Gerking, 1978). Only by evaluating the population dynamics of a species can the long-term effect of environmental perturbations be fully assessed. The research proposed herein examines these processes to ascertain the effects, if any, of ELF.

2. Production

Production will be estimated and compared between the control site and the ELF site for 2 to 3 fish species. To assess the effects of perturbation on a fish community, the quantitative performance of the community must be estimated. LeCren (1972) stated that production may be the best parameter to describe the overall quantitative performance of a fish community because it integrates both growth and survival characteristics of that fish community. Production, defined as the elaboration of tissue over time regardless of the ultimate fate of that tissue (Ivlev 1966), is also a sensitive indicator of energy utilization and storage by organisms. Changes in production from a perturbation could indicate that the energy flow in an ecosystem is altered and this could disrupt the efficient functioning of the ecosystem.

Dace and sculpins will be randomly sampled at all stations on a seasonal basis. Sampling will be done using square meter kick nets,

seines and visual observation (Whitworth and Schmidt 1980). All fish collected using square meter kick nets and seines will be weighed and measured for total length. Fish will also be marked using fin clipping and cold or silver nitrate branding for concurrent mark and recapture population estimates.

Brook trout will be randomly sampled at all stations on a seasonal basis and will only be used in production studies if an adequate sampling method can be found. Sampling methods will include block seining, and visual observation (Whitworth and Schmidt 1980). Fish collected by seining will be weighed and total length measured. Brook trout will also be marked using either individually numbered tags, fin clipping or branding for a concurrent mark-recapture population estimate.

Population estimates will be calculated using catch per area estimators, (meter square kick samplers), catch per effort estimators (visual observations and seining) and mark-recapture techniques. Catch per effort and catch per area estimations are direct estimations. Population estimates from mark-recapture techniques will be calculated using a Schnable multiple mark-recapture modification as expressed by:

$$N = \frac{\Sigma C M}{\Sigma R}$$

where N = population estimate

C = total catches
M = total marks
R = total recaptures

These data will then be combined with mean weight estimates to calculate mean biomass (B) which is needed to calculate production.

Production will be estimated using the numerical method described by Ricker (1946) and modified by Chapman (1978), which is:

$$P = G_x \; \Delta t \; \bar{B}$$

where G_x is the instantaneous rate of growth, calculated using the formula:

$$G_x = (\ln \bar{w}_2 - \ln \bar{w}_1)/\Delta t$$

where w_1 and w_2 = mean weights of fish at the beginning and the end of a time interval (t). Seasonal biomass estimates will be made and overage biomass during each interval between each estimate will be calculated using the formula:

$$\bar{B} = \frac{B_1 [\exp (G_x - z^1)\Delta t - 1]}{(G_x - z^1)\Delta t} \quad \text{if } G_x > z_1$$

$$\text{OR}$$

$$\bar{B} = \frac{B_1 [\exp (-(z^1 - G_x)\Delta t)]}{(z^1 - G_x)\Delta t} \quad \text{if } G_x < z^1$$

where B_1 = initial biomass. z^1, the instantaneous rate of decrease will be calculated using the formula

$$z^1 = -(\ln N_2 - \ln N_1)/\Delta t$$

A graphical analysis of production a described by Allen (1951) will also be used to verify production estimates.

Both production and population estimates will be compared between the control and ELF sites using either students T test or Mann-Whitney U Tests depending on normality of the data.

3. Reproduction and Growth

Although production is a sensitive overall indicator of quantitative performance, it does not show which qualitative factors are responsible for a community's performance. The qualitative factors integrated into production are growth, recruitment, fecundity, condition and mortality. Thus, to adequately understand how a potential perturbation affects a community, the dynamic factors integrated into production must be studied concurrently with production.

Dace and sculpins will have vital statistics examined concurrently with the production section. Dace and sculpins from the production study will also have pectoral fin rays removed for aging. At two other randomly selected non- production stations at both sites, dace and sculpins will be collected and preserved in 10% formalin for later otolith removal which will verify fin ray aging techniques. These fish will be measured for total length and weighed. A subsample of the preserved fish will be measured for total length and weighed before and after preservation to provide a correction factor to counter the well known effects of formalin on fish weight.

Brook trout will also be measured for total length, weighed and scales taken for aging (from the posterior of the pectoral fin) during the production study. This species will only be used if an adequate sampling technique is found.

Length-weight regression relationships will be calculated using log transformed data to insure linearity. Age determination will be estimated using scale techniques for brook trout, and fin ray sectioning for dace and sculpins (Bagenal 1978). Growth will be examined, using both mean size at annulus and through back-calculated growth. Back calculations of growth will be estimated using body-scale regression relationships. Growth rates will be further estimated known length-weight fish from marked individuals in the production study. These fish will be monitored through time for growth.

Condition factors will be determined using length-weight data for all stations. Condition factors will be calculated using:

$$K = \frac{W}{L^3} \quad \text{or} \quad K = \frac{W(10^5)}{L^3}$$

where w = weight and L = length.

Growth rates and condition factors will be compared between sites using either Student's t-tests or Mann-Whitney U tests depending upon normality of the data.

Mortality will be assessed for 2 to 3 fish species using the same marked individuals as used in the population mark-recapture studies as expressed by $M = 1 - \frac{M_1}{M_2}$ where M_x = fish marked in age class at time X, and using numerical abundance of each age group over time following the methods described by Ricker (1975). Mortality will be additionally examined using a graphical analysis as described by

Allen (1951). Mean mortality rates by age will be compared between sites using either Student's t-tests or Mann-Whitney U-tests depending on the normality of the data.

Fish reproductive fitness will be assessed using two fecundity estimates and recruitment on 2 to 3 fish species. Fecundity, defined as the number of ripening eggs in the female prior to the next spawning period (Bagenal 1978), will be determined using: 1) standard volumetric techniques and will be expressed as $F=aL^b$ where F=fecundity, L= total length, and a and b = species specific constants; and 2) GSI (Gonadotrophic Somatic Index) which is expressed as GSI = (Gonadal wet weight/ total body wet weight- gonadal wet weight) x 100. Dace and sculpins collected for otolith examination and brook trout from the 2 non-production stations will be used for GSI estimation. Brook trout will be used only if an adequate sampling technique can be found. GSI will be determined seasonally, and fecundity estimates will be done on fish collected at non-production stations a month before and after spawning using the techniques discussed in the production section.

Recruitment, defined as the supply of fish that become available at some particular stage in their life history (Everhart et al. 1975), will be investigated. This process is a sensitive indicator of hatchability and development problems and integrates these processes. These data will be acquired through the mortality study at both sites. Any physical deformities seen in the fish will be recorded.

Fecundity, and recruitment will be compared between the control and ELF sites using either Student's t-test or Mann-

Whitney U-tests depending on the normality of the data.

4. Food Assimilation and Habitat Utilization

Production and its integrated factors provide an excellent indication of how a fish community is performing but do not directly show how well that community is utilizing its available resources of habitat and food. If a perturbation is causing a change in the optimal utilization of resources the perturbation could be a underlying reason for any shift in production as well as other factors related to production. Feeding and food habits are sensitive indicators of food availability thus can be a measure of secondary production changes by a perturbation. Ayermeier (1982) stated that changes in the quantity and quality of a food resource are expected to elicit changes in the food utilization by fish. Thus, to food habits must be examined how a perturbation may affect a community. Not only can food habits indicate the quality and quantity of a food resource, but these data can also be used to examine the efficiency of the resource use. One can calculate food consumption by combining the temporal use of food with food habits. Calculated food consumption can then be extrapolated to total energy consumed by the fish community. These data, secondary production estimates, and fish production estimates can be used to give an estimate of the efficiency of energy use and the amount of energy transferred between trophic levels. In conclusion, to adequately assess the effects of a perturbation, the changes in food utilization and energy transfer under the stresses must be measured.

Habitat and its utilization, the other resource that is partitioned in a stream, may also influenced by a perturbation. Alterations of the environment may cause the habitat to be used inefficiently. An inefficient use of a resource may then place a stress on the organism causing energy for growth and reproduction to be transferred to organismal maintenance. Productivity would probably also change as an overall result of this stress.

Food habits will be analyzed on a seasonal basis using gut samples from the control and ELF sites. Live sculpins and brook trout will have gut contents sampled using stomach tubes (Van Den Avyle and Roosell (1980), stomach pumps and pulse gastric salvage (Foster 1977). Live dace gut contents will be sampled using back flushing (Baker and Fraser 1976). Gut samples will be taken from representative individuals studied in the production component of the work. Stomach samples will also be examined from fish that were preserved for fecundity analysis. Brook trout will be used only if an adequate sampling technique can be found.

Food items in the guts will be identified to the lowest taxonomic level, total length and head capsule width measured, percent volume estimated and each food taxa weighed (either spun wet weight or dry weight). Partially digested food items will have length and weight estimated from regression equations of hardened body parts to length and weight. Mean food habits for each fish species will be calculated by age and length, and compared, using Kruskel-Wallis contingency table analysis, between the control and ELF sites.

Two electivity indices will be calculated to determine which food items are actively selected regardless of availability,: 1) Ivlev's index (1961) which is $\frac{S-b}{S+b}$ where S = percentage by weight of a food organisms in the gut contents adb = percentage representation of the same organism in the environment and 2) Strasse's linear index $L = R_i - P_i$ where R_i = Proportion of a food item in the gut and P_i = proportion of a food item in the environment. Both indices range from +1 to -1. Electricity indices will be compared by Mann-Whitney U tests between the control and ELF sites.

Food consumption will be determined seasonally using the methodology as described by Staples (1975) at the 2 non-production stations. A random sample of fish will be captured every 4 hours over two consecutive 24 hr periods; one-half sample will be killed upon capture, the other half will be left 4 hours in a food-free tank before being killed. The daily ration (DR) is calculated by $DR = \sum_{1}^{6} (\bar{w}_2 - \bar{w}_{16})$ where $\bar{w}_2$ is the mean weight of the gut contents of the fish sampled at the end of any 4 hr period and $\bar{w}_{16}$ is the mean weight of the gut contents of the tank held field fish. Food consumption will be compared between the control and the ELF site using Mann-Whitney U-tests.

Habitat utilization will be estimated for 2 to 3 fish species. Habitat will be measured along with population estimate measurements using the same fish. Brook trout will be related with respect to habitat on a per site rather than per area basis owing to sampling techniques (block seining).

Brook trout will be related to habitat by mapping sites, using the population and condition statistics and the area of each substrate type. Dace and sculpins will be directly related to macro & micro habitat on a per area basis owing to sampling technique restrictions (meter square kack netting). Substrate will be recorded in dace and sculpin sampling using a paired meter stick transect method. This technique, is measured to the near arm using two (adjacent to sampling area) perpendicular transects along which each substrate particle. Current velocity will be measured using either a Gurley pygmy curent meter or a salt tablet technique described by Rabeni and Minshall (1977). Correlation analysis and multivariate regression models will show which habitat characteristics are important to the fish community (Binns, 1979). Mapped habitat characteristics and actual measured habitat for dace and sculpins are the Xiji's in the model and the vital statistics (population, condition etc.) will be the Y's in the models. All data will be tested for normality and will be transformed if necessary. Models will be compared using F^* for regression models (Neter and Wasserman 1974) and Mann-Whitney U tests for correlation coefficients between the control and ELF sites. Brook trout will only be used if an adequate sample technique is found.

Habitat will be analyzed using two microtechniques for 2 to 3 fish species at the same stations as for the production study. The first technique was previously described for dace and sculpins. The second technique is a working approach used by Faush (1978) where all fish are noted in locations. (Locations flagged for habitat and velocity measurements), and fish sized estimated using

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substrate keys flagged. Locations will be measured physically for 1) current velocity, 2) cover type, 3) substrate size and 4) fish depth. Fish that were previously individually marked will be investigated as to habitat preference and location. Correlation, and multivariant regression analysis will be done for microhabitat data analysis. Comparison between the control site and the ELF site will be done using the F^* test (Netter and Wasserman 1974) for regression models and Mann-Whitney U tests for correlation analysis.

5. Fish Movements

The effects of ELF on migration and homing ability will be assessed with 2 to 3 species of fish.

Many authors suggest that fish may use electromagnetic fields as an additional cue for migration and homing. Wisconsin and Ohio studies on Ray billed gulls (Southern 1973) and homing pigeons (Grave 1974) have demonstrated alterations in bird orientation owing to electromagnetic radiation. These data indicate that migration and homing abilities may be sensitive indicators of ELF's effects (if any) on fish.

Fish migration is critical to optimal utilization of spawning sites. If the optimal spawning habitat cannot be found owing to interference, the long term persistance of a species may be endangered.

Migration will be estimated with directional fyke nets. They act as weirs across the stream and intercept upstream or downstream movement. A pair of nets will be used (one hoop net facing upstream and the other facing downstream) at the control site and the

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ELF site. The nets will be set for a one week period each season and will be checked daily. Fish will be weighed, measured and tagged with individually numbered tags, fin clips or brands. The fish will be put below the traps in the direction of travel. Migration rates will be calculated in catch per effort statistics (i.e. fish per net day). These rates for each fish species will be compared between the control and ELF sites using either t-Tests or Mann-Whitney U tests depending on the raw data.

The homing ability of two to three fish species will be determined quarterly (Gerking, 1950, 1953, 1959) at the control and ELF sites. Ten fish of each species will be removed from a non-production site, individually marked and released at 2 m distances downstream and upstream from the capture site. Subsequent visual observation and netting of their home area will quantify their homing activity. Homing percentages will be calculated and compared between the control and the ELF site using either arcsin transform data for t-tests or Mann-Whitney U-tests.

6. Parasite Loads

The parasitic and pathogenic loads of fish will be determined at both the control and ELF site.

Previously, we have stated that perturbations exert fish. If fish are not damaged directly by these perturbations they may have weakened resistance and be vulnerable to attacks by infectious agents (Allison et al. 1977). The relationship between stress and parasitism at the individual, population and ecosystem levels of biological organization has been well documented (Esch et al.

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1979).

Previous studies of aquatic organisms (Eure, 1976; Esch et al.
1979; Pojmanska et al. 1980) have demonstrated that perturbation of
aquatic environments may lead to: 1) an impoverishment of the parasite
fauna in general; 2) an increase or decrease in the parasite community
diversity; 3) elimination of a parasite species; 4) breaking down of
host specificity and infection of new hosts; 5) and a possible
increase the pathogenic effect of parasites on their hosts owing to
the increase of their number and deterioration of fish condition.
Specific objectives are to investigate and to compare these
host-parasite relationships as well as the parasite parameters
mentioned above. Parasitological data may indicate indirectly that
the lotic environments studied are undergoing stress.

Parasitic and pathogenic loads of fish will be done using the
same fish sampled for fecundity and during food habit gut studies
at the non-production stations. Fish will be preserved in 10%
formalin, measured and analyzed in the lab for parasites.

To date, the study has established a taxonomic base of
parasites that infect fishes in the proposed area (15 genera: 3
protozoans, 2 monogenetic trematodes, 2 digenetic trematodes, 2
cestodes, 4 nematodes, 1 acanthocophalan and 1 crustacean. The
distribution, infection rates, species diversity and fish condition
of parasites are being calculated. These calculations will be
analyzed between the control and ELF sites using either t-tests or
Mann-Whitney U-tests. Correlation analysis between parasitism
parameters and fish condition factors to establish stress
relationships will also be done.

Overall Statistical Analyses

There is a wide array of parameters that can be measured when the physical monitoring, periphyton, aquatic insect and fish components are integrated. Some data will be represented graphically, other data will be analyzed in the measured form, and some data will be incorporated into indices which will then be treated as individual parameters. The following section presents the general statistical design that will be utilized in the proposed research and the specific statistical manipulations that are required for several required comparisons.

The basic design is a replicated two-way analysis of variance with habitat (riffles and pools) and location (control and experimental) constituting the treatments. The replication will vary between N=2 and N=5, depending on the task. Duplicate samples are sufficient for the water chemistry monitoring due to turbulent mixing of the water. Five replicates are required for the artifical substrate samples containing benthic organisms. This results from a tradeoff between the number needed to compensate for intersample variation and the number of samples that can be physically processed. It is impossible to estimate the desired sample size at this time by conventional methods since the

minimum deviation in ($\bar{X}i-\mu$) that is acceptable has not yet been determined.

The ecologically interesting comparisons involve contrasts between habitats within a site (riffle versus pools) and contrasts

between sites within a habitat (experimental versus controls). The parameters of ecological interest vary with the task. In general, the colonization rates and the growth forms have been determined from the 1982 field work. The time intervals for sampling have been chosen such that the sampling unit will have reached equilibrium with the ambient conditions. Parameter estimates are obtained from point samples at equilibrium. These are the data to be utilized in the standard two-way analysis of variance. The parameters for the periphyton and aquatic insects involve species numbers, total numerical densities, total biomass and aggregate indicies of diversity, redundancy and evenness. Other parameters involving chemical composition, productivity, stream drift and migration (distance moved) are specific to individual subtasks.

Transformations are likely to be needed to fulfill the assumptions of normality and independence of means and variances. The specific transform functions will not be known until data are available. Growth and biomass data most likely will require a logarithmic transformation and the percent composition by species will require an arcsin transformation.

A second general design will involve a two-way analysis of variance with time series data within each cell. The main treatments are again habitats (riffles and pools) and locations (experimental and control). The experiments involve repeated sampling involving growth rates (fish) and degradation rates (leaf packs). These experiments are measuring processes rather than state variables. The exact form of the analysis will depend upon

the shape of the observed function. If the curves are consistantly a given function (linear, exponential, etc.) across treatments, a covariance analysis can be utilized. The number of replications available will also constrain the choice of statistical analysis.

The third basic analysis involves analyses of associations. There are approximately 12-15 chemical and physical parameters which are routinely monitored for water quality. These data will be summarized in tabular form as means and standard errors for descriptive purposes. Seasonal patterns will be obtained from these data. Correlation matricies will be utilized to screen for associations of parameters that appear to fluctuate in concert with seasonal patterns of biological events. A subset of these physical parameters (most likely phosphorus, nitrate nitrogen, temperature and dissolved oxygen) will be utilized in a multiple regression analysis.

The purpose of this analysis is to partition the seasonal variation in the dependent parameter (species diversity, biomass, etc.) into components associated with each independent parameter (NO_3, P, temperature, etc.). The partial regression coefficients will be compared between habitats (riffles and pools) and between locations (experimental and control). The specifics as to which parameters will be included in the regression analysis can not be determined until the field data are available.

There are a number of isolated uses of statistical analysis that are specific to subtasks. Regression analysis is used to estimate dry weights from live weights for the leaf pack experiments. Similar estimators are utilized for length-weight

relationships with fish. Parametric student's t-test and non parametric Mann-Whitney U tests will be used for specific comparisons; e.g., electively indices and local microhabitat distributions for fish. The extent of the use of these nested comparisons will only be apparent when a full years' data is available for the next annual report.

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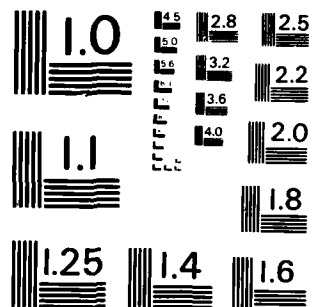
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Work Plan and Progress TO DATE

Element 1 - Paired Plot Selection

Synopsis: Preliminary plots have been selected on
each of two separate stream systems. The
general stream systems included in this
study were selected early in July of
1982. Final selection of a control plot
will be completed in the early summer of
1983.

Contributing Staff - Thomas M. Burton, Associate Professor (PI)
Richard W. Merritt, Associate Professor (PI)
R. Jean Stout, Research Associate (PI)
William W. Taylor, Assistant Professor (PI)

Progress to Date - After numerous visits to stream sites within
the proposed ELF corridor, we selected the
following experimental and control plots
in 1982.

Stream Order	Experimental Site	Control Site
2	Schwartz Creek (T44N, R28W, Sect. 10)	McGregor Creek (T44N, R28W, Sect. 25)
3	West Branch Escanaba: Site 1 (T44N; R27W, Sect. 19)	West Branch Escanaba: Site 2 (T44N, R27W, Sect. 11)

These study sites were selected based on various stream
parameters such as: order, channel depth and width, bottom type,
faunal assemblages, riparian vegetation and other watershed
characteristics. Two additional study sites (Ford 1-Expt. Site
(R29W, T43N), Ford 2-Control Site (R29W, T43N) were chosen as 4th
order stream comparisons.

Permission was obtained from either state agencies or private
land owners to conduct research on the study sites.

Element 2 - Inventory Physical Characteristics of Stream Sites

Synopsis - Determine general physical charac-
teristics of the two study streams
including width, depth, shape, and
velocity. Determine particle size
distribution for bottom sediments in
riffle and pools of each stream as well
as length of pools and riffles
(riffle/pool length ratio). Determine
riparian vegetation along the stream
margin including species composition and

dominance of forest vegetation and
canopy coverage of stream.

Contributing Staff - R. Jean Stout, Research Associate (PI)
                     Michael O'Malley, Field Reseach Tech. II
                     William Taft, Field Research Tech. II

Progress to Date -

1. Particle size distribution. Benthic substrate samples
    taken from riffle and pool areas in the Ford, West Branch
    of the Escanada, Schwartz and McGregor Creeks (3
    replicates from pools and 3 replicates from riffles in
    the four streams and rivers). Total: 24 samples, each
    sieved into 7 size categories.

2. Widths and depths of the streams. Widths and depths were
    taken in the Ford and W. B. Escanaba rivers.

3. Velocity was recorded over 24-hour periods in the Ford
    and W. B. Escanaba during the drift studies. Velocity and
    depths recorded in both rivers at 20 points along cross-
    sections for later determination of discharge.

4. Riparian vegetation was characterized along the Ford,
    West Branch of the Escanaba and along Schwartz Creek
    early in the season.

Element 3 - Establish and Conduct Ambient Monitoring Program

Synopsis - The ambient monitoring program has
consisted of daily sampling of all water
chemistry parameters for each plot as
well as continuous monitoring of several
ambient parameters (D.O., pH, etc.)
detailed in the proposal. The automatic
monitoring stations were ordered in
July, 1982 but will not be installed
until final plot selection after the
exact right-of-way is known. All other
monitoring began August, 1982 and
continued on a daily basis (5 day/week)
through October, 1982. They will be
reduced to monthly samples from
November 1, 1982 to April 1, 1983 when
daily sampling will resume.

Contributing Staff - Thomas M. Burton, Associate Professor (PI)
                     Michael J. O'Malley, Field Research Tech. II
                     Undergraduate Research Aide as needed.

Progress to Date -   Continuous monitoring stations have been
                      ordered but cannot be installed until final
                      ELF corridors are agreed upon. Specific
                      corridor location will not be available to
                      within 100 yards until December, 1982. Thus,
                      monitoring stations will be installed in the
                      spring of 1983 as soon as snow melt allows.
                      Interim sampling consisted of back-
                      ground chemical sampling at six stations on
                      four streams within the ecological impact
                      area in Dickinson County. Most intensive
                      sampling was conducted at two sites on the
                      Ford River with an average of 3.2 samples per
                      week from August 1- November 1, 1982. The
                      other 4 sites had an average of 2.3 samples
                      per week collected. In addition, water
                      samples were taken at two hour intervals for
                      one 24 hour cycle from the Ford River and
                      West Branch of the Escanaba. Samples were
                      analyzed for PH, specific conductance,
                      turbidity, dissolved oxygen, alkalinity, and
                      hardness in the field using standard methods.
                      Separate samples were frozen for later
                      analyses of all other chemical constituents
                      listed in the proposal. These samples will be
                      analyzed during the winter in East Lansing.

Element 4 - Effects of Exposure Period on Periphytic Colonization of Artificial Substrates

        Synopsis - Artificial glass slide substrates have
                   been placed in pools and riffles of the
                   two study streams. Substrates were
                   removed daily to assess the impact of
                   exposure period on species composition,
                   numbers of individuals, diversity,
                   biomass accumulation, and chlorophyll $\underline{a}$/
                   phaeophytin $\underline{a}$ production.

Contributing Staff - Thomas M. Burton, Associate Professor (PI)
                     Mark Oemke, Research Associate
                     Michael J. O'Malley, Field Research Tech. II
                     Undergraduate Research Aide as needed

Progress to Date-

Long Term Study -   Slides were collected at designated intervals
to determine colonization rates and species diversity changes
through time. Two sites on the Ford River (F.S. II- 2 mi W of
Ralph and F.S. I- 8.5 mi W of Ralph) were selected as well as the
West Branch of the Escanaba below the junction of Schwartz Creek &
Flat Rock Creek (W.B.E.I.). Slides were collected on days 4, 7,
14, and every 7 days thereafter for 8 weeks from each site.

Changes in colonization rates and species composition with changing light penetration and water temperatures were assessed with this procedure utilizing 3 sets of experiments. The first set was initiated on Aug 3rd to run for 8 weeks, the second was initiated on Aug 17 to run for 8 weeks and the trial on September 1st to run for 8 weeks at Ford site I and Ford Site II. This sampling design should provide detailed information on the fluctuations in algal populations over the time period. A total of 108 slides from each of the 3 sites on the Ford will be examined and 40 from W.B.E.I.

Short Term Study -   Several slides were collected during the early stages of colonization in order to more accurately measure early diversity changes and colonization rates. Day 4, 5, 6, 9, 10, 11 were chosen and samples collected from F.S.I. and F.S.II. on these dates.

Effects of colonization rate on biomass accumulation and chlorophyll a/phaeophytin a production were determined at Ford site I, Ford site II, and at the West Branch of the Escanaba Site I. Three replicates for biomass and 3 for chlorophyll a/ phaeophytin a were collected every 4 days for 28 days from each site. Two of these 28-day studies were conducted.

All samples from the above activities were preserved and will be analyzed during the winter and spring.

Element 5 - Compare Periphyton Communities on Natural Substrates with those on Artificial Substrates

Synopsis - As a check on efficiency of sampling, natural substrates were sampled and compared to communities on artificial substrates. This comparison was done intensively during the first month of the study and at periodic intervals throughout the remainder of the study.

Contributing Staff - Thomas M. Burton, Associate Professor (PI)
Mark Oemke, Research Associate
Michael J. O'Malley, Field Research Tech. II
Unknown Graduate Research Assistant
Undergraduate Research Aides as needed

Progress to Date -   Natural substrates were sampled during all periods of the study at all 6 sites. Natural periphyton was removed from all available substrates in the streams. Scrapings from rock surfaces, algal or macrophyte sequeezings, sediment samples, and micellaneous collections from wood or twigs were made. Field samples were preserved for analyses during the winter. Natural substrate communities will be compared to communities on slides (as described in Element 4 and 6).

<u>Element 6 - Monitoring of Species Composition, Numbers, Diversity,
Biomass production, cell volume, and Chlorophyll a/
Phaeophytin a Production for periphyton</u>

Synopsis - Routine monitoring of the parameters
listed above began on August 1, 1982 for
the streams to be studied. This
monitoring will be moved to specific
paired plots after they are selected. It
will continue throughout the course of
the study.

Contributing Staff - Thomas M. Burton, Associate Professor (PI)
Mark Oemke, Research Associate
Michael J. O'Malley, Field Research Tech. II
Unknown Graduate Research Assistant
Undergraduate Research Aide as needed

<u>Progress to Date</u> - Initial monitoring studies were conducted as
part of the colonization experiments (Element 4) for Ford site I,
Ford site II, and West Branch Escanaba I. Based on preliminary
observations from these studies, a 12-day exposure interval was
selected for chlorophyll a/phaeophytin a and periphyton biomass
accumulation studies. Four replicates for each determination were
collected from Schwartz Creek, West Branch Escanaba Site II, and
McGregor Creek after 12-day exposure periods 9/14, 9/16, 9/28,
10/10, and 10/22/82. After the 28-day colonization rate studies
were completed on F.S.I, F.S.II, and W.B.E.I, 12-day monitoring
studies were initiated at these sites also, with removal on
10/22/82. After 10/22/82, 28-day exposure periods was initiated at
all sites as part of the winter sampling regime.

The 12 day monitoring studies for chlorophyll a and biomass
were conducted simultaneously with 8, 12, 16, and 20 day studies
of species abundance, diversity and cell volume. These studies
will be continued at the 28-day interval throughout the winter.

All samples were preserved and will be analyzed during the
winter.

<u>Element 7 - Stream Invertebrate Collection and Identification</u>

Synopsis - Collect and identify invertebrates
present and prepare reference collection
for species checklist and as aide to
routine identification for each stream
site.

Contributing Staff - R. Merritt, Associate Professor (PI)
J. Stout, Research Associate (PI)
W. Taft, Field Research Tech. II
K. Webb, Graduate Research Assistant

Progress to Date – Qualitative samples of macroinvertebrates were taken at all stream sites to establish a species list of the fauna and determine the relative abundance or major groups. These collections, in addition to those specimens collected in Elements 8-14, will be sorted and identified during the winter. From some preliminary work, we have identified the following taxa.

ORDER	FAMILY	GENUS AND/OR SPECIES
Plecoptera	Perlidae	Neophasgonophora capitata (Pictet)
		Acroneuria lycorias (Newman)
Odonata	Gomphidae	Ophiogomphus sp.
	Libellulidae	
Ephemeroptera	Caenidae	Tricorythodes sp.
	Ephemerellidae	Ephemerella cornuta Morgan
		E. deficiens Morgan
		E. simplex McDunnough
	Baetidae	Baetis spp.
	Heptageniidae	Heptagenia spp.
Magaloptera	Corydalidae	Nigronia sp.
Trichoptera	Brachycentridae	Brachycentrus sp.
	Glossosomatidae	Glossosoma sp.
	Limnephilidae	Pycnopsyche sp.
	Philopotamidae	Dolophilodes sp.
Diptera	Chironomidae	
	Athericidae	Atherix

Element 8 – Effects of Exposure Period on Invertebrate Colonization of Artificial Substrates

Synopsis – Exposed artificial substrates in stream for different time periods (e.g., 3, 7, 10 days) to assess maximum colonization time. Analysis of benthic samples will be completed during winter months.

Contributing Staff – J. Stout, Research Associate (PI)
R. Merritt, Associate Professor (PI)
W. Taft, Field Research Tech. II
Undergraduate Research Aide as needed

Progress to Date – Sample baskets were placed in pool riffle sections of the Ford and West Branch of the Escanaba. Two replicates from riffles in each of the two rivers were collected after 4, 7, and 14 day's immersion.
TOTAL: 24 samples.

Element 9 - Species Richness and Biomass of Stream Macro-Invertebrates in Riffles and Pools from Artificial Substrates

Synopsis - By means of artificial substrate samples and selected quantitative stream benthic samplers, we will determine the species richness and biomass per unit area at selected stream sites. Analysis of benthic samples will be conducted during the winter.

Contributing Staff - R. Merritt, Associate Professor (PI)
J. Stout, Research Associate (PI)
T. Burton, Associate Professor (PI)
W. Taft, Field Research Tech. II
K. Webb, Graduate Assistant
Undergraduate Research Aide as needed

Progress to Date - Samples were collected in five replicates from the Ford River and the West Branch of Escanaba River after 21 and 54 days in the water. (We used the same collection schedule as for fresh leafpacks). Total: 20 samples.

Element 10 - Movement Patterns of Selected Aquatic Invertebrates

Synopsis - By means of mark-recapture techniques, selected grazers and predators of the grazers will be collected, marked and released for later recapture (1, 2, 3, 7 days after release) to determine distances and directions travelled for the individuals. Various marking colors will be used according to day of marking. Analyses of data will occur during fall and winter.

Contributing Staff - J. Stout, Research Associate (PI)
R. Merritt, Associate Professor (PI)
M. Ufford, Graduate Research Assistant

Progress to Date - Population Estimate of Crayfish (Orconectes sp.)

A riffle area 45 m long, 16 m wide, 0.5 mi upstream from Ford Site I was chosen as the location for a population estimate of crayfish. The initial capture was made on 24 August and three (3) subsequent mark-recapture sessions occurred on 28 August, 31 August and 4 September. Crayfish were captured by kick-seine and held in buckets until they could be sexed, measured and marked with enamel paint. Each day was assigned a different color so the recapture history of individuals could be tracked. The animals were segregated by location of capture (left, center, right) and returned each day to the appropriate part of the riffle. The data are summarized:

DATE	# MARKED	# RECAPTURED	% RECAPTURED
24 August	36	-	-
28 August	136	3	2.2
31 August	127	25	19.7
04 September	97	25	25.3

Lincoln Index Population Estimate:

28 August:	1620*
31 August:	776
04 Sept. :	545

*Questionable estimate because recapture success was too small (2.2%)

Element 11 - Colonization Patterns and Processing by Invertebrates on Autumnal Freshly Fallen Leaves

Synopsis - Examine the species diversity, species richness, abundance and biomass of stream macroinvertebrates on natural leaf litter accumulations in the stream. This task will also involve the collection and storage of leaves for leaf litter experiments in Fall of 1983.

Contributing Staff - J. Stout, Research Associate (PI)
T. Burton, Associate Professor (PI)
R. Merritt, Associate Professor (PI)
K. Webb, Graduate Research Assistant
W. Taft, Field Research Tech. II

Progress to Date -

A. Fresh leaf inputs (two experiments)
1. Freshly picked alder leaves. Put leafpacks in streams 5 August. Leafpacks consisted of 10 leaves per pack. Three replicates were collected from the Ford and West Branch of the Escanaba after 3, 6, 12, 25 and 54 days of immersion. Total: 30 samples. Loss in leaf area, dry weight loss computed; colonizing insects on leaves preserved in 70% alcohol.

2. Freshly picked alder leaves. Put leafpacks in streams 27 August. Leafpacks consisted of 10 leaves per pack. Five replicates were collected from the Ford, West Branch of the Escanaba and Schwartz Creek after 3, 9, 27 and 54 day's immersion. Total: 60 samples.

3. Regression of leaf area to dry weight for 200 leaves done $(r^2 = 0.85)$

B. Freshly fallen and then dried leaves (two experiments)
collected from parachutes daily.
1. Alder leaves. Put in streams 21 September. Five replicates
of 10 leaves each collected from Ford, West Branch of
Escanaba and Schwartz Creek after 3, 9 and 27 day's
immersion. Total: 45 samples.

2. White Birch leaves. Put in streams 1 October. Five
replicates of 10 leaves each collected from Ford, West
Branch of Escanaba and Schwartz Creek ater 2, 9 and 27
day's immersion. Total: 45 samples.

(Note: All leaf packs will continue to be collected monthly from
November of 1982 to June of 1983).

Element 12 - Drift Patterns of Aquatic Invertebrates

Synopsis - Determine behavioral drift patterns of
major macroinvertebrates utilizing drift
nets. These studies will be run for
selected time periods and at selected
intervals. Identification of major fauna
will be done during winter.

Drift studies were not proposed as
part of our revised proposal, owing to
budget changes. We will do drift
studies, however, but they will be used
only as corollary data for fish
production studies.

Contributing Staff - R. Merritt, Associate Professor (PI)
J. Stout, Research Associate (PI)
K. Webb, Graduate Research Assistant
W. Taft, Field Research Tech. II

Progress to Date - Drift samples were collected in triplicate at
the head and tail ends of the riffle sites on the West Branch of
Escanaba River at 2 hr. intervals from 4.00 p.m., 9 September,
4:00 p.m., 10 September. Sampling period was 15 minutes. Total: 72
samples. Drift samples were collected in triplicate at the head
and tail ends of a riffle site at Ford I at 2 hr. intervals until
after dark and then at 3 hr. intervals, beginning at 4:00 p.m. on
13 September and ending at 3:00 p.m. on 14 September. Total: 54
samples.

(Note: water chemistries, velocity, temperature, leaf drift
samples were taken during both of the 24-hour runs)

Element 13 - Leaf Litter Processing Experiments Using Natural Leaf Packs and Cages

Synopsis - Lab-constructed leaf litter packs were placed out in the streams during the fall and litter breakdown rates were assessed on a weekly and monthly basis. Once major shredder organisms are identified, caged leaf packs containing selected macroinvertebrates will be placed out and leaf degradation rates will be followed.

Contributing Staff - R. Merritt, Associate Professor (PI)
T. Burton, Associate Professor (PI)
J. Stout, Research Associate (PI)
W. Taft, Field Research Tech. II
K. Webb, Graduate Research Assistant
Undergraduate Research Aide as needed

Progress to Date - Aquatic insects from the freshly fallen leaves (see Element 11) will be identified this winter. After the major shredder organisms are identified, the caged leaf pack experiments will be initiated (late summer of 1983).

One m square leaf litter collecting traps were constructed and 10 traps were placed above the water level in the Ford, West Branch of the Escanaba and Schwartz Creek for a total of 40 traps. Leaves falling into the traps were collected 11 times from 6 September to 20 October.

Amount and type of leaves in the drift were assayed, using drifts nets submersed in water for 15 minutes for each sampling period for the Ford, West Branch of the Escanaba and Schwartz Creek.

Element 14 - Feeding Activity of Grazer Populations

Synopsis - Major grazing macroinvertebrates were identified. Feeding strategies and resource partitioning for some species were followed.

Contributing Staff - R. Merritt, Associate Professor (PI)
T. Burton, Associate Professor (PI)
M. Oemke, Research Associate
K. Webb, Graduate Research Assistant

Progress to Date - Major grazing taxa were identified in both the West Branch of the Escanaba River (WBE) and the Ford River. The caddisflies (Glossosoma sp. and Protoptila tenebrosa were abundant in both rivers, as were the mayflies Baetis spp. Ephemerella spp. and Heptagenia sp.. The caddifly, Leucotrichia pictipes, was extremely abundant in at least two separate sites on the Ford River but was virtually absent from WBE.

Preliminary qualitative analyses of gut contents were performed. Only Leucotrichia and Protoptila were found to utilize periphyton as their major food resource. Although it had already pupated when studies began, Glossosoma is known from previous studies to feed almost exclusively on periphyton. The mayflies were more generalized in their feeding habits, with a greater proportion of unidentified material (detritus) in their guts. A 24-hour series of samples of Leucotrichia was taken for a more detailed quantitative and qualitative analysis of its feeding habits.

Element 15 - Fish Species Composition, Relative Abundance, and Habitat Relationships

Synposis -   The fish communities in a section of the two streams under or adjacent to the ELF system (experimental) and a control site were sampled using nets and visual underwater observations to determine species composition of the fish fauna, relative abundance, and habitat preference.

Contributing Staff - William W. Taylor, Assistant Professor (PI)
David Gesl, Graduate Research Assistant
Unknown Field Research Tech. II
Undergraduate Research Aides as needed

Progress to Date -   Fish surveys of the Ford and Escanaba River systems were conducted in August and September 1982. Both stream systems were relatively infertile and exhibited low species richness. In total, 14 species were collected (Table 1) Longnose dace (Rhinichthys cataractae) was the most abundant fish species; however, their distribution was limited to the larger riffle areas of the streams. Sculpins (Cottus cognatus and C. bairdi) were the second most abundant species. They were common in riffle areas and present at lower densities in the slow portions of the streams. Blacknose dace (Rhinichthys atratulus) and burbot (Lota lota) were found at low densities in certain portions of riffles. Brook trout (Salvelinus fontinalis) were generally found to be scarce in the larger rivers but concentrated in smaller, cooler tributaries.

Table 1 -   Species list of fish captured in the Ford and Escanaba
            river systems during August and September 1982.

Family Catastomidae
  1. Catastomus comersoni   White sucker

Family Cottidae
  1. Cottus bairdi   Mottled sculpin
  2. Cottus cognatus   Slimy sculpin

Family Cyprinidae
  1. Chrosomus eos   Northern redbelly dace
  2. Chrosomus erythrogaster   Southern redbelly dace
  3. Rhinichthys atractulus   Blacknose dace
  4. Rhinichthys cataratae   Longnose dace
  5. Semotilus corporalis

Family Gadidae
  1. Lota lota   Burbot

Family Percidae
  1. Etheostoma nigrum   Johnny darter
  2. Etheostoma blennoides   Green side darter

Family Salmonidae
  1. Salmo trutta   Brown trout
  2. Salvelinus fontinalis   Brook trout

Family Umbridae
  1. Umbra limi   Central Mudminnow

Element 16 - Assessment of Equipment Efficiency for Capture of
             Selected Fish Species

            Synopsis - Fish capture efficiency using nets were
                        evaluated by comparing net capture with
                        visual underwater observation for the
                        study sites. In addition, capture
                        efficiency were compared to electro-
                        fishing capture efficiency for adjacent
                        streams not included in the study
                        (introduction of additional electro-
                        magnetic radiation in the study areas
                        will be avoided).

  Contributing Staff - William W. Taylor, Assistant Professor (PI)
                       David Gesl, Graduate Research Assistant
                       Unknown Field Research Tech. II
                       Undergraduate Research Aides as needed

Progress to Date -   Netting techniques proved to be difficult
and generally inefficient for most species. Kick seining was
effective in capturing sculpins and dace in riffles. Brook trout
were collected using a directional fyke net which was effective
between dusk and dawn. Minnow traps were used with limited
success. New methods of fish capture are currently being
evaluated.

Element 17 - Age-Length-Weight Relationships; Growth, Fecundity, Survival, and Distribution of Selected Fish Species

Synopsis -   Some basis vital statistics of selected
species (sculpin, Cottus bairdi, and
brook trout, Salvelinus fontinalis) were
determined from specimens captured with
seines and by visual observation in the
control and experimental sites.

Contributing Staff - William W. Taylor, Assistant Professor (PI)
David Gesl, Graduate Research Assistant,
Unkown
Field Research Tech. II
Undergraduate Research Aide as needed

Progress to Date -   The vital statistics of the various fish
populations enountered were examined. Length-weight-age and growth
analyses are currently being performed on the individuals
captured. Brook trout fecundity estimates will be made in October--
early November during spawning.

Element 18 - Diurnal Food Habits and Consumption Rates of Selected Fish Species

Synopsis -   Food habits of selected fish species
(sculpin and brook trout) and
consumption rates were determined in the
control and experimental sites for the
study streams.

Contributing Staff - William W. Taylor, Assistant Professor (PI)
David Gesl, Graduate Research Assistant
Unknown
Field Research Tech. II
Undergraduate Research Aide as needed

Progress to Date -   Food habits of brook trout, sculpins and
longnose dace were examined in the Ford and Escanaba river
systems. Brook trout feed primarily on terrestrial organisms with
crayfish, mayfly nymphs and water bugs being less common. Sculpins
and dace consumed principally mayfly and stonefly nymphs and
caddisfly larvae.

Element 19 - Mark - Recapture Studies of Sculpin

Synopsis - The sculpin, <u>Cottus</u> <u>bairdi</u>, will be collected from the control and experimental sites of each study stream with nets, marked and released. This mark-recapture procedure will continue until reasonable population estimates are established and will be repeated seasonally.

Contributing Staff - William W. Taylor, Assistant Professor (PI)
David Gesl, Graduate Research Assistant
Unknown
Field Research Tech. II

<u>Progress to Date</u> - Mark-recapture studies were performed on longnose dace and sculpins in a riffle area in the Ford River approximately 9.5 miles west of Ralph, Michigan. Initial population estimates (Peterson technique) for dace and sculpin were 580 ± 147 and 200 ± 59, respectively.

Element 20 - Studies of Patterns of Development from Egg to Adult for Selected Fish Species

Synopsis - The development pattern of brook trout and sculpin will be determined by visual observation and specimen collection and study for the control and experimental sites.

Contributing Staff - William W. Taylor, Assistant Professor (PI)
David Gesl, Gradaute Research Assistant
Unknown Field Research Tech. II
Undergraduate Research Aide as needed

<u>Progress to Date</u> - Brook trout development patterns will be studied upon spawning in October.

Element 21 - Parasite Loads of Selected Fish Species

Synopsis - The internal and external parasites of selected fish species were determined by collection of fish and laboratory examination of the kinds and numbers of parasites present for both the control and experimental sites.

Contributing Staff - Patrick Muzzall, Assistant Professor

Progress to Date – Individuals of 14 fish species representing six families have been collected from the Ford and Escanaba Rivers and their tributaries. Fishes have been examined alive on site or have been preserved in formalin and necropsied later in the laboratory. Emphasis has been on the examination of the blacknose dace, longnose dace, mottled sculpin, and brook trout. Organs being examined are eyes, gills, flesh, swim bladder, viscera, and digestive tract. The prevalence and mean number of the parasite species found are being calculated. Parasite species found and identified to date are: Trematoda-Crepidostomum sp. (anterior intestine of brook trout), Acanthocephala-Neoechinorhynchus sp. (intestine of brook trout), Copepoda-Salmincola edwardsii (gills and fins of brook trout) and Myxosgoridea-Myxobolus sp. (gills of longnose dace). Parasite species diversity for each fish will be calculated and the parasite species diversity for the fish species will be composed.

Element 22 – Data Analysis and Report Writing

Synopsis – Data for each task will be entered into the data management system developed by Eco-tech, Inc. as soon as it is collected or analyses are done. Reports will be submitted as required by contract.

Contributing Staff – All persons listed in Elements 1-21.

Progress to Date – Data management forms and statistical packages have been designed for our data collection, tabulation and analyses.

CONCLUSIONS

In the Basic Proposal Period (07/01/82-10/31/82) we have
accomplished the following:

1. Selected the experimental and control sites. (Originally, our
   Flat Rock Run Club site on the West Branch of the Escanaba
   River was to be the experimental site for a 3rd and 4th-order
   stream. Late in the field season of 1982, the ELF corridor
   site was altered. Rather than crossing the Escanaba, the new
   proposed corridor crossed the Ford River. We walked the Ford
   River and found an appropriate experimental site, which is
   approximately 2 mi downstream from our Ford I site and 4 mi
   upstream from our Ford II site. The new site, 0.1 mi above
   the confluence of Turner Creek and the Ford River, will be
   used as our experimental site for a 4th-order stream
   system).

2. Ordered the equipment necessary to initiate the aquatic
   research;

3. Secured on-site housing accomodations;

4. Hired personnel to work on the project;

5. Identified and described major physical and chemical
   characteristics of chosen stream sites;

6. Collected and identified some of the major fanual assemblages
   within the stream sites (algae, aquatic insects, fish);

7. Initiated the following experiments:
   1) Leaf litter colonization and degradation studies
   2) Periphyton colonization studies
   3) Aquatic insect colonization studies
   4) Movement and drift patterns of aquatic macro-
      invertebrates
   5) Mark-recapture studies of selected fish species

8. Examined parasite loads of selected stream animals

DA
FILM